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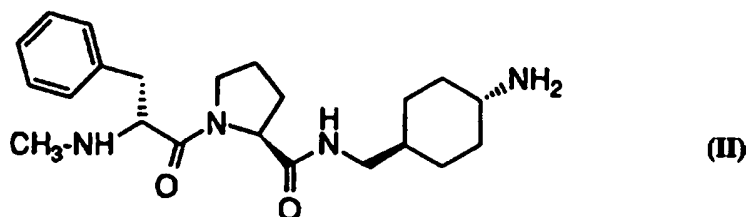
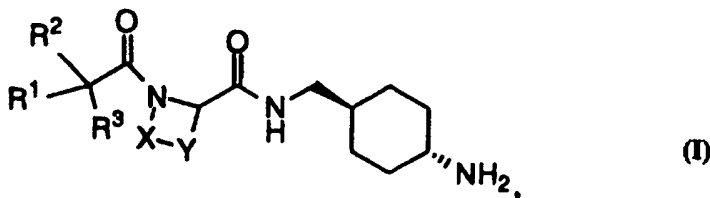
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(54) Title: THROMBIN INHIBITORS

(57) Abstract

Compounds of the invention have structure (I) for example formula (II). These compounds inhibit thrombin and associated thrombosis.



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TITLE OF THE INVENTION
THROMBIN INHIBITORS

BACKGROUND OF THE INVENTION

5 Thrombin is a serine protease present in blood plasma in the form of a precursor, prothrombin. Thrombin plays a central role in the mechanism of blood coagulation by converting the solution plasma protein, fibrinogen, into insoluble fibrin.

 Edwards *et al.* *J. Amer. Chem. Soc.* (1992) vol. 114, pp. 1854-63, describes peptidyl α -ketobenzoxazoles which are reversible inhibitors of the serine proteases human leukocyte elastase and porcine pancreatic elastase.

 European Publication 363 284 describes analogs of peptidase substrates in which the nitrogen atom of the scissile amide group of the substrate peptide has been replaced by hydrogen or a substituted carbonyl moiety.

15 Australian Publication 86245677 also describes peptidase inhibitors having an activated electrophilic ketone moiety such as fluoromethylene ketone or α -keto carboxyl derivatives.

20 Thrombin inhibitors described in prior publications contain sidechains of arginine and lysine. These structures show low selectivity for thrombin over other trypsin-like enzymes. Some of them show toxicity of hypotension and liver toxicity.

25 SUMMARY OF THE INVENTION

 These compounds show selectivity for thrombin over trypsin and other trypsin-like enzymes and have oral bioavailability. Trypsin-like enzymes (such as trypsin, thrombin, factor xa, kallikrein, plasmin, urokinase, and plasminogen activator) are serine dependent enzymes that catalyze hydrolysis at arginyl and lysyl peptide bonds.

30 The invention includes a composition for inhibiting loss of blood platelets, inhibiting formation of blood platelet aggregates, inhibiting formation of fibrin, inhibiting thrombus formation, and inhibiting embolus formation in a mammal, comprising a compound of

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the invention in a pharmaceutically acceptable carrier. These compositions may optionally include anticoagulants, antiplatelet agents, and thrombolytic agents. The compositions can be added to blood, blood products, or mammalian organs in order to effect the desired inhibitions.

5 The invention also includes a composition for preventing or treating unstable angina, refractory angina, myocardial infarction, transient ischemic attacks, atrial fibrillation, thrombotic stroke, embolic stroke, deep vein thrombosis, disseminated intravascular coagulation, ocular build up of fibrin, and reocclusion or restenosis of recanalized
10 vessels, in a mammal, comprising a compound of the invention in a pharmaceutically acceptable carrier. These compositions may optionally include anticoagulants, antiplatelet agents, and thrombolytic agents.

 The invention also includes a method for reducing the thrombogenicity of a surface in a mammal by attaching to the surface,
15 either covalently or noncovalently, a compound of the invention.

 Some abbreviations that may appear in this application are as follows.

ABBREVIATIONS

20

Designation

BOC (Boc)

CBZ (Cbz)

TBS (TBDMS)

Protecting Group

t-butyloxycarbonyl

benzyloxycarbonyl(carbobenzoxo)

t-butyl-dimethylsilyl

Activating Group

HBT(HOBT or HOBt)

1-hydroxybenzotriazole hydrate

Designation

BOP reagent

BOP-Cl

Coupling Reagent

benzotriazol-1-yloxytris-
(dimethylamino)phosphonium

hexafluorophosphate

bis(2-oxo-3-oxazolidinyl)phosphinic
chloride

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EDC 1-ethyl-3-(3-dimethylaminopropyl)
carbodiimide hydrochloride

Other

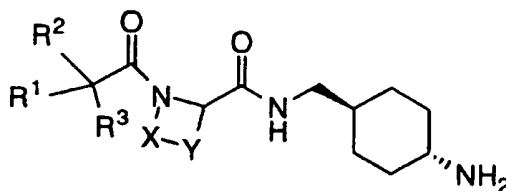
(BOC) ₂ O (BOC ₂ O)	di-t-butyl dicarbonate
n-Bu ₄ N ⁺ F ⁻	tetrabutyl ammonium fluoride
nBuLi (n-Buli)	n-butyllithium
DMF	dimethylformamide
Et ₃ N	triethylamine
EtOAc	ethyl acetate
TFA	trifluoroacetic acid
DMAP	dimethylaminopyridine
DME	dimethoxyethane
LDA	lithium diisopropylamide
THF	tetrahydrofuran

Amino Acid

Ile	Isoleucine
Phe	Phenylalanine
Pro	Proline
Ala	Alanine
Val	Valine

DETAILED DESCRIPTION OF THE INVENTION

5 Compounds of the invention have the following structure:



wherein

R¹ and R² are independently

10 hydrogen,

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- phenyl,
mono- or di-halogenated phenyl,
naphthyl,
biphenyl,
5 a 5- to 7- membered mono- or bicyclic heterocyclic ring or
bicyclic heterocyclic ring system any ring of which may be
saturated or unsaturated, and which consists of carbon atoms and
from one to three heteroatoms selected from the group consisting
of N, O and S,
10 C₁₋₄ alkyl,
branched C₁₋₄ alkyl,
C₃₋₇ cycloalkyl,
C₅₋₁₂ bicyclic alkyl,
C₁₁₋₁₆ tricyclic alkyl,
15 (CH₂)_nR⁴,
CH(R⁴)₂, wherein R⁴ is the same or different,
CH(R⁴)(OR⁴), provided that "OR⁴" is not OCOOH or
OCONHR⁵,
(CH₂)_nOR⁴, provided that "OR⁴" is not OCOOH or
20 OCONHR⁵ or
R² may be joined with R¹ to form a four- to seven membered
carbon ring in which zero to two carbon atoms may be substituted
with heteroatoms independently selected from the list N, O, and S,

25 where n is 1, 2, 3 or 4;

R³ is

- 30 H,
N(R¹)₂, wherein R¹ is the same or different,
R¹OCONH, provided R¹ is not hydrogen,
R¹CONH,
(CH₂)_pOH, where p is 0, 1, 2, 3 or 4,
R¹SO₂NH, provided R¹ is not hydrogen, or

- 5 -

$(R^1)_mNCONH$, where m is 1 or 2, wherein R^1 is the same or different;

R^4 is

- 5 phenyl,
 mono- or di-halogenated phenyl,
 naphthyl,
 biphenyl,
 a 5- to 7- membered mono- or bicyclic heterocyclic ring or
10 bicyclic heterocyclic ring system any ring of which may be
 saturated or unsaturated, and which consists of carbon atoms and
 from one to three heteroatoms selected from the group consisting
 of N, O and S,
 $COOR^5$,
15 $CONHR^5$,
 C_{1-4} alkyl,
 branched C_{1-4} alkyl,
 C_{3-7} cycloalkyl,
 C_{5-12} bicyclic alkyl, or
20 C_{11-16} tricyclic alkyl;

R^5 is

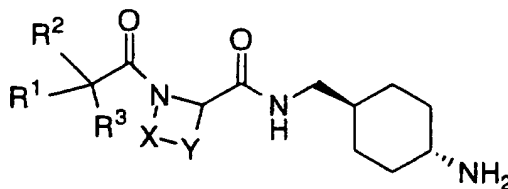
- hydrogen,
 C_{1-4} alkyl, or
25 branched C_{1-4} alkyl;

X is $(CH_2)_q$ where q is 1 or 2; or
 NR^1CH_2 ; and

- 30 Y is SCH_2 , or
 $(CH_2)_r$ where r is 1 or 2.

In one class, the compounds have the following structure:

- 6 -



wherein

R^1 and R^2 are independently

- 5 hydrogen,
 phenyl,
 mono- or di-halogenated phenyl,
 naphthyl,
 biphenyl,
- 10 a 5- to 7- membered mono- or bicyclic heterocyclic ring or
 bicyclic heterocyclic ring system any ring of which may be
 saturated or unsaturated, and which consists of carbon atoms and
 from one to three heteroatoms selected from the group consisting
 of N, O and S,
- 15 C_{1-4} alkyl,
 branched C_{1-4} alkyl,
 C_{3-7} cycloalkyl,
 C_{5-12} bicyclic alkyl,
 C_{11-16} tricyclic alkyl,
- 20 $(CH_2)_n R^4$,
 $CH(R^4)_2$, wherein R^4 is the same or different,
 $CH(R^4)(OR^4)$, provided that " OR^4 " is not $OCOOH$ or
 $OCONHR^5$,
 $(CH_2)_n OR^4$, provided that " OR^4 " is not $OCOOH$ or
- 25 $OCONHR^5$ or
 R^2 may be joined with R^1 to form a four- to seven membered
 carbon ring in which zero to two carbon atoms may be substituted
 with heteroatoms independently selected from the list N, O, and S,
- 30 where n is 1, 2, 3 or 4;

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R³ is

- 5 H,
 N(R¹)₂, wherein R¹ is the same or different,
 R¹OCONH,
 R¹CONH,
 (CH₂)_pOH, where p is 0, 1, 2, 3 or 4,
 R¹SO₂NH, or
10 (R¹)_mNCONH, where m is 1 or 2, wherein R¹ is the same or
 different;

R⁴ is

- 15 phenyl,
 mono- or di-halogenated phenyl,
 naphthyl,
 biphenyl,
 a 5- to 7- membered mono- or bicyclic heterocyclic ring or
 bicyclic heterocyclic ring system any ring of which may be
20 saturated or unsaturated, and which consists of carbon atoms and
 from one to three heteroatoms selected from the group consisting
 of N, O and S,
 C₁₋₄ alkyl,
 branched C₁₋₄ alkyl,
25 C₃₋₇ cycloalkyl,
 C₅₋₁₂ bicyclic alkyl, or
 C₁₁₋₁₆ tricyclic alkyl;

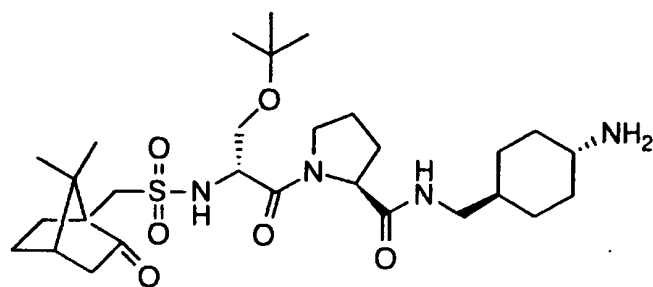
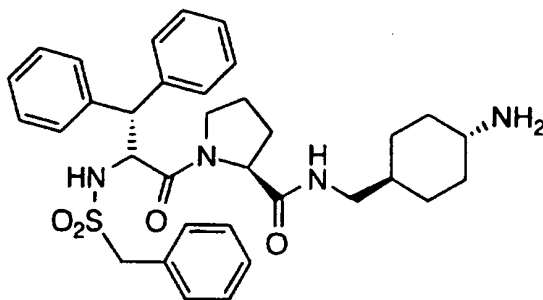
- 30 X is (CH₂)_q where q is 1 or 2; or
 NR¹CH₂; and

- Y is SCH₂, or
 (CH₂)_r where r is 1 or 2.

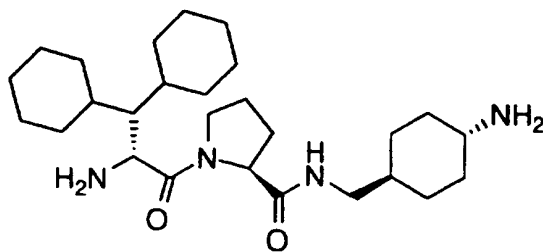
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In one subclass of compounds of the invention, X is $(CH_2)_q$; q is 1; Y is $(CH_2)_r$; and r is 1 or 2. Specific embodiments of this class include

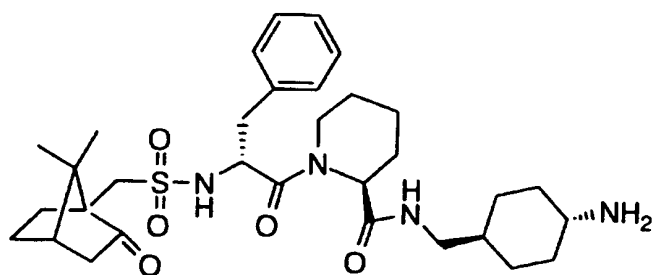
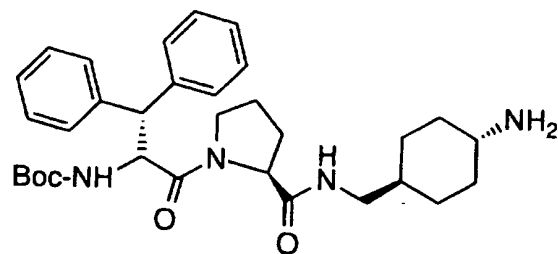
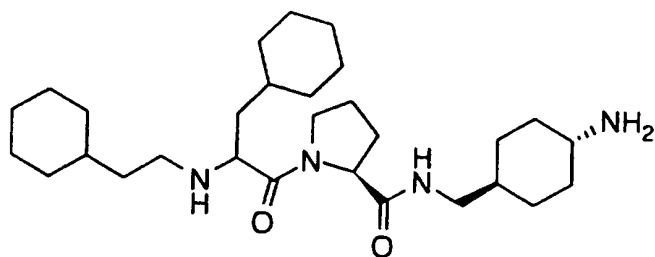
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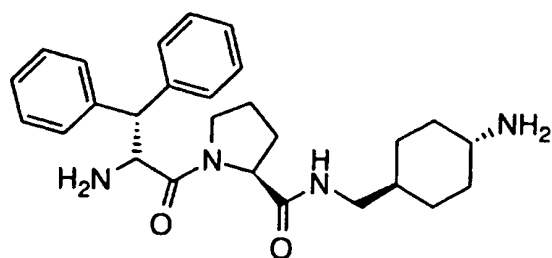
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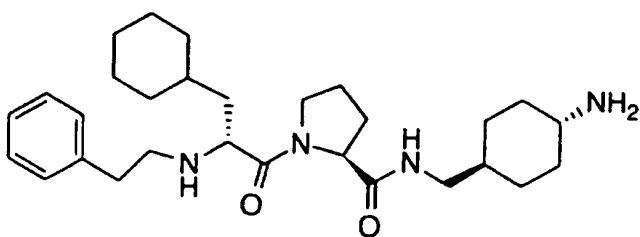
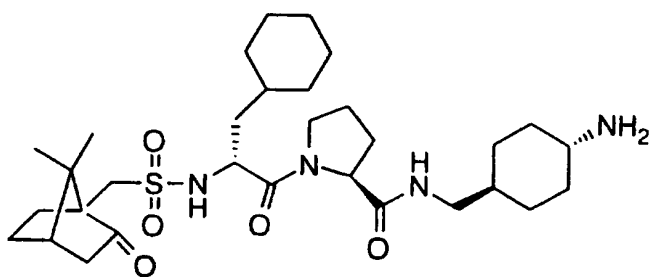
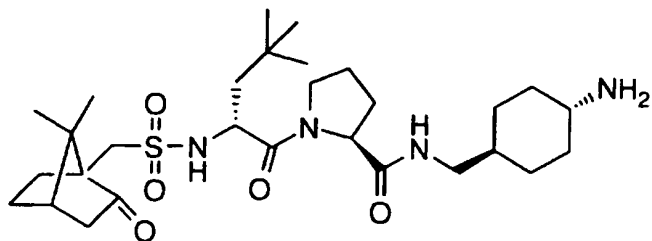
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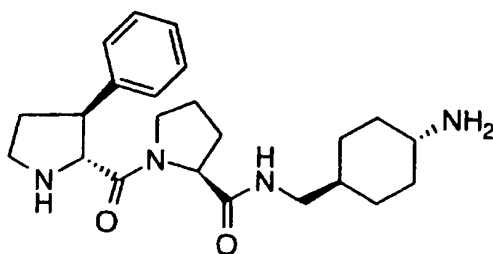
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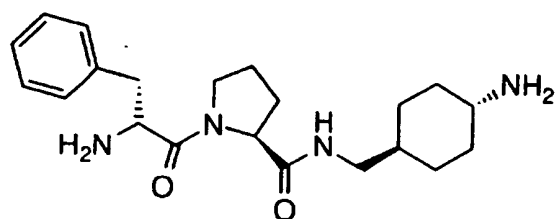
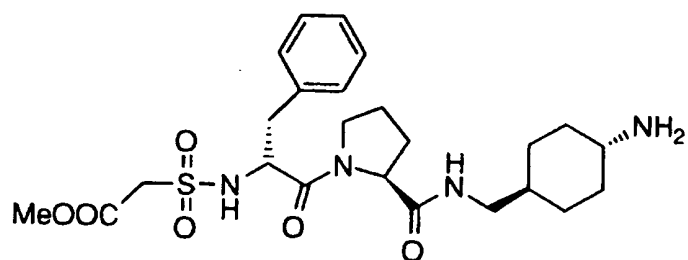
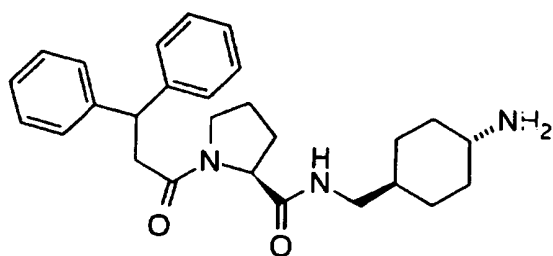
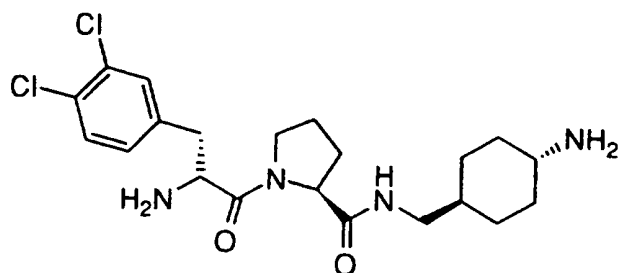
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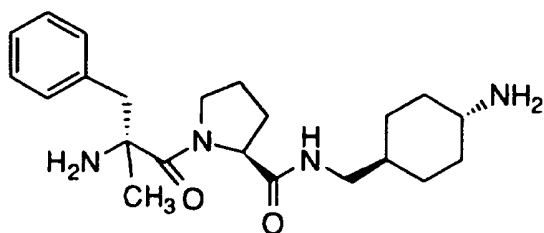
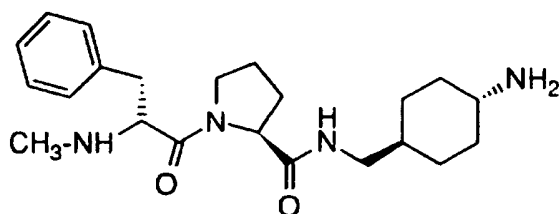
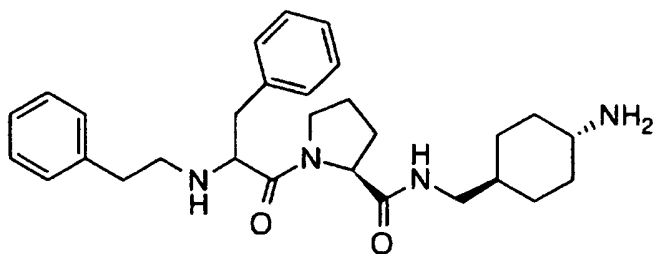
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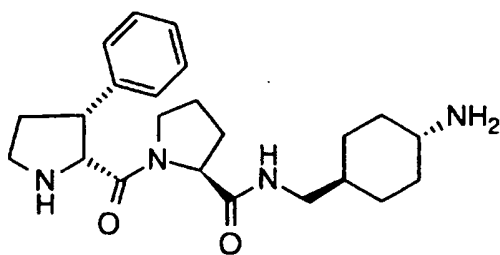
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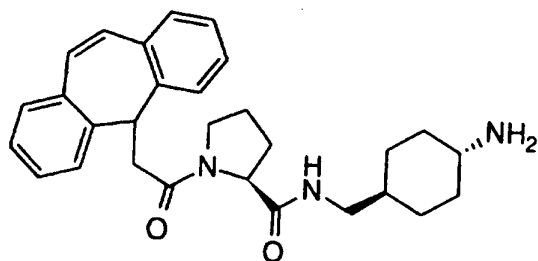
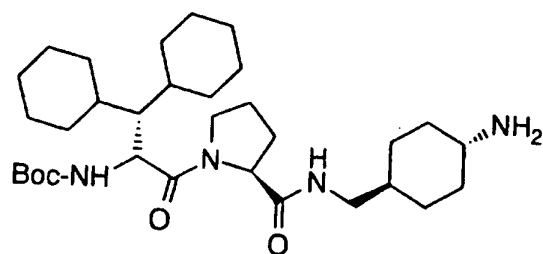
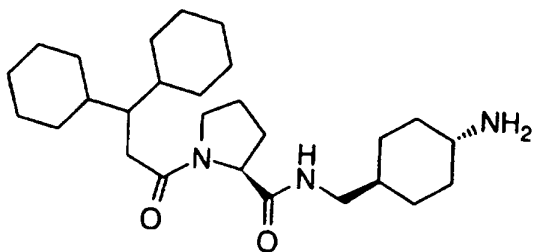
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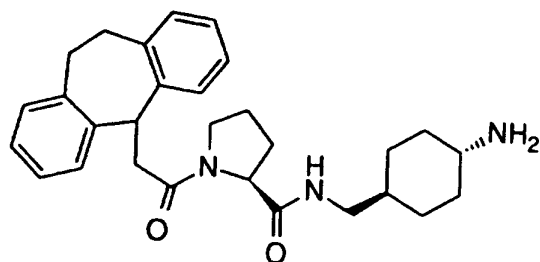
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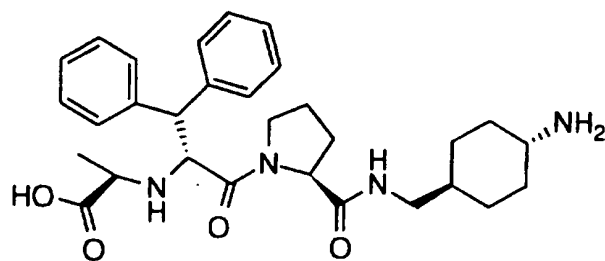
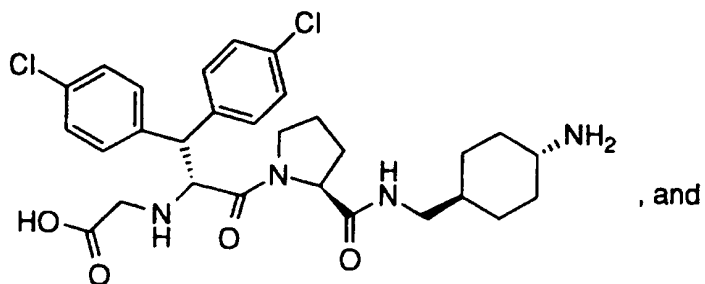
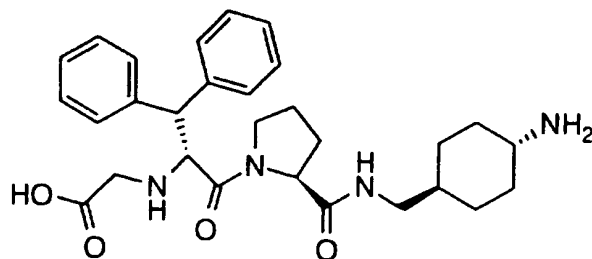
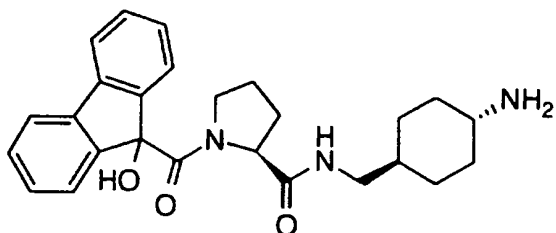
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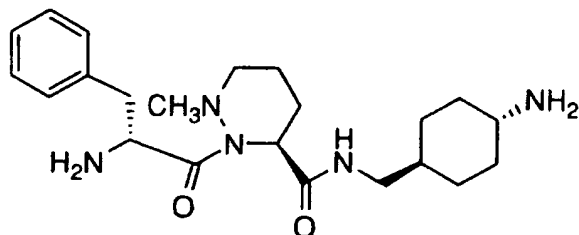
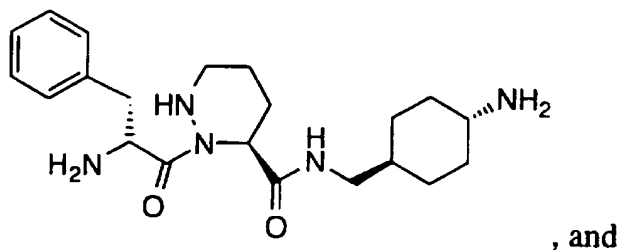


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In another subclass of compounds of the invention, X is
 NR¹CH₂; R¹ is hydrogen or C₁₋₄ alkyl; Y is (CH₂)_r; and r is 2. Specific
 5 emboments of this class include

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- 5 The compounds of the present invention, may have asymmetric centers and occur as racemates, racemic mixtures and as individual diastereomers, or enantiomers with all isomeric forms being included in the present invention. A racemate or racemic mixture does not imply a 50:50 mixture of stereoisomers.
- 10 When any variable occurs more than one time in any constituent or in formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.
- 15 As used herein except where noted, "alkyl" is intended to include both branched- and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms (Me is methyl, Et is ethyl, Pr is propyl, Bu is butyl); "alkoxy" represents an alkyl group of indicated number of carbon atoms attached through an oxygen bridge;
- 20 "Halo", as used herein, means fluoro, chloro, bromo and iodo; and "counterion" is used to represent a small, single negatively-charged species, such as chloride, bromide, hydroxide, acetate, trifluoroacetate,

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perchlorate, nitrate, benzoate, maleate, tartrate, hemitartrate, benzene sulfonate, and the like.

The term heterocycle or heterocyclic, as used herein except where noted, represents a stable 5- to 7-membered mono- or bicyclic or
5 stable 7- to 10-membered bicyclic heterocyclic ring system any ring of which may be saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S, and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom
10 may optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic elements include piperidinyl, piperazinyl, 2-oxopiperazinyl,
15 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, pyrrolyl, 4-piperidinyl, pyrrolidinyl, pyrazolyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, quinuclidinyl, isothiazolidinyl, indolyl,
20 quinolinyl, isoquinolinyl, benzimidazolyl, thiadiazoyl, benzopyranyl, benzothiazolyl, benzoxazolyl, furyl, tetrahydrofuryl, tetrahydropyranyl, thienyl, benzothienyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, and oxadiazolyl. Morpholino is the same as morpholinyl.

25 The pharmaceutically-acceptable salts of the compounds of Formula I (in the form of water- or oil-soluble or dispersible products) include the conventional non-toxic salts or the quaternary ammonium salts which are formed, e.g., from inorganic or organic acids or bases. Examples of such acid addition salts include acetate, adipate, alginate,
30 aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate,

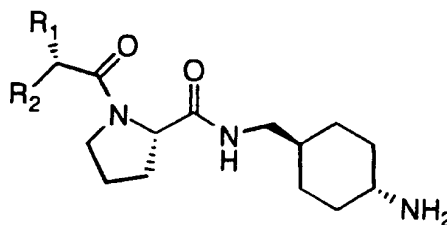
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methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium
5 salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups may be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and
10 butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others.

Amide couplings used to form the compounds of this
15 invention are typically performed by the carbodiimide method with reagents such as dicyclohexylcarbodiimide, or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. Other methods of forming the amide or peptide bond include, but are not limited to the synthetic routes via an acid chloride, azide, mixed anhydride or activated ester. Typically,
20 solution phase amide coupling are performed, but solid-phase synthesis by classical Merrifield techniques may be employed instead. The addition and removal of one or more protecting groups is also typical practice.

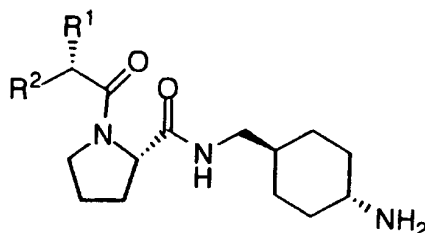
The compounds shown in the tables below are exemplary
25 compounds of the present invention:

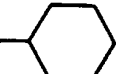
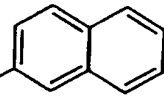





- 18 -

TABLE I

R_1	R_2	Ki vs Thrombin (nM)
PhCH ₂	H	680
C ₆ H ₁₁ CH ₂	H	150
3,4-Cl ₂ -PhCH ₂	NH ₂	1.5
PhCH ₂	NH ₂	3.4
		47
3,4-Cl ₂ -PhCH ₂	CH ₃ NH	2.9
		152
PhCH ₂	CH ₃ SO ₂ NH	17
3,4-Cl ₂ -PhCH ₂	H	414

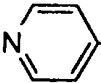





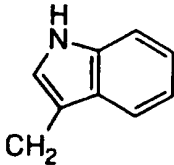
- 19 -

TABLE 1 CONTINUED

R ₂	R ₁	Ki (nM) vs thrombin
H	H	>10000
CH ₃ NH	H ₂ C- 	2.3
NH ₂	H ₂ C- 	27
NH ₂	CH ₂ - 	30
OH	CH ₂ - 	63
AcNH	CH ₂ - 	170
BocNH	CH ₂ - 	9.4
CbzNH	CH ₂ - 	3.7

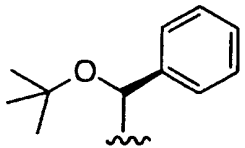
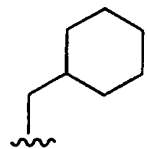
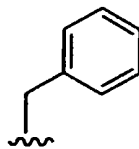
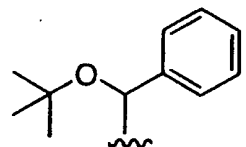
- 20 -

TABLE 1 (CONT'D)

R ²	R ¹	Ki (nM)vs thrombin)
 -CH ₂ OCONH	CH ₂ - 	15
BocNH	 - 	6.8
BocNH	CH ₂ - 	12
BocNH	CH ₂ CH ₂ - 	50
BocNH	CH ₂ O-tBu	6
CbzNH	CH ₂ -tBu	2.3
CbzNH		16

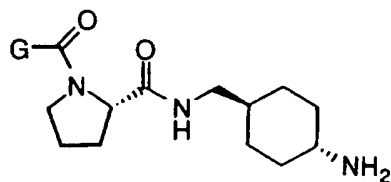
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TABLE 1 (CONTD)

R2	R1	Ki (nM) vs Thrombin
Boc-NH		8.0
CF ₃ CH ₂ SO ₂ NH		3.0
PhCH ₂ CH ₂ NH		4.0
Boc-NH		200

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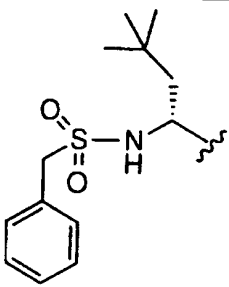
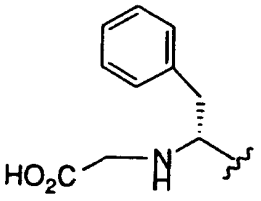
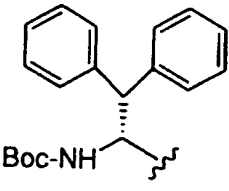
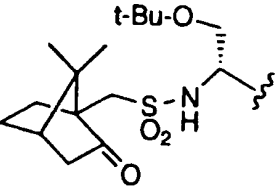
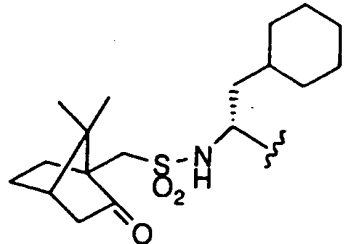
TABLE 2



G	Ki (nM vs thrombin)
<p>Chemical structure of G: A chiral center with a phenyl group, a benzyl group, and a 2-aminoethyl group.</p>	0.12
<p>Chemical structure of G: A chiral center with a phenyl group, a benzyl group, and a 3-aminopropyl group.</p>	2.0
<p>Chemical structure of G: A chiral center with a phenyl group, a benzyl group, and a 2-(benzenesulfonylamino)ethyl group.</p>	0.4
<p>Chemical structure of G: A chiral center with a phenyl group, a benzyl group, and a 2-(benzenesulfonylamino)ethyl group.</p>	0.0025

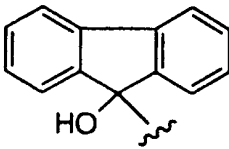
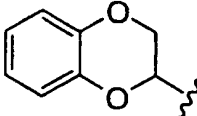
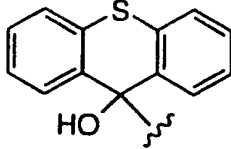
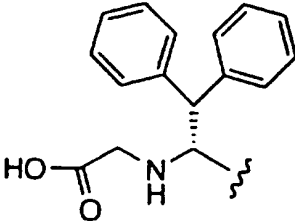
- 23 -

TABLE 2 (CONT'D)

G	Ki (nM vs thrombin)
	0.4
	1.3
	0.102
	0.05
	0.1

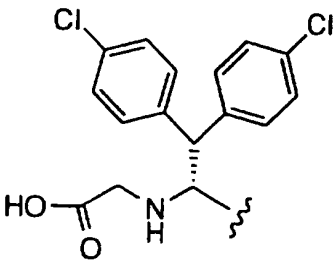
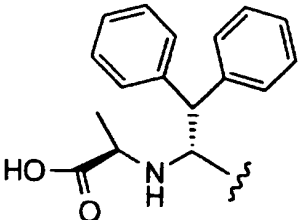
- 24 -

TABLE 2 (CONT'D)

G	Ki (nM)
	1.5
	55
	3
	0.17

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TABLE 2 (CONT'D)

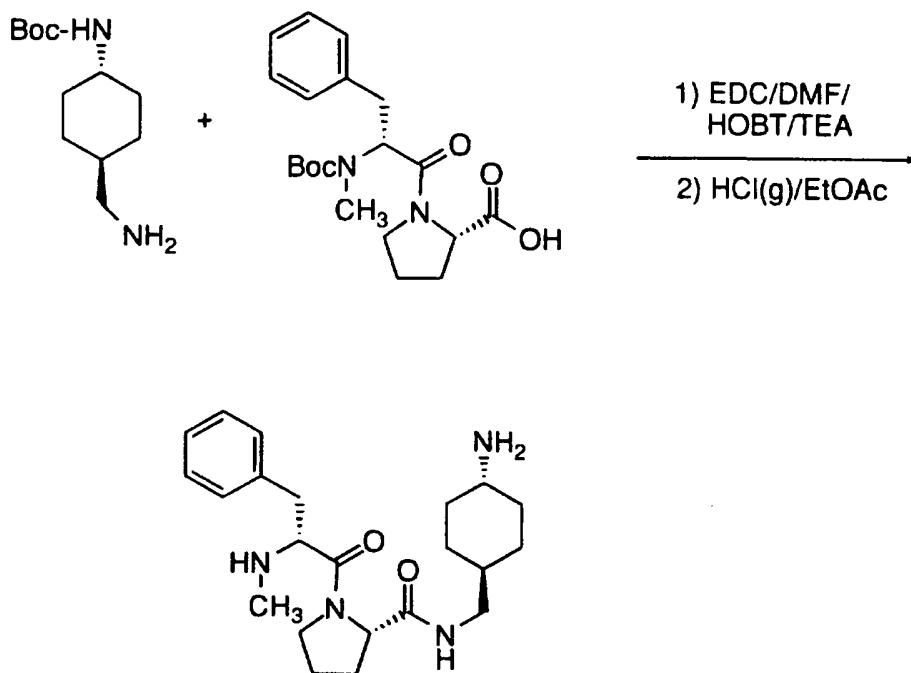
<u>G</u>	<u>Ki (nM)</u>
	0.13
	0.05

5

The following synthetic routes can be used to prepare compounds of the invention. Using method I (as exemplified by example 1), *trans*-4-*t*-butoxycarbonylamino-cyclohexyl-methanamine (1-3) is coupled to a protected dipeptide such as N-methyl-D-phenylalanyl-L-proline using standard amide coupling procedures. The BOC protecting groups can then be removed using a strong acid such as HCl gas.

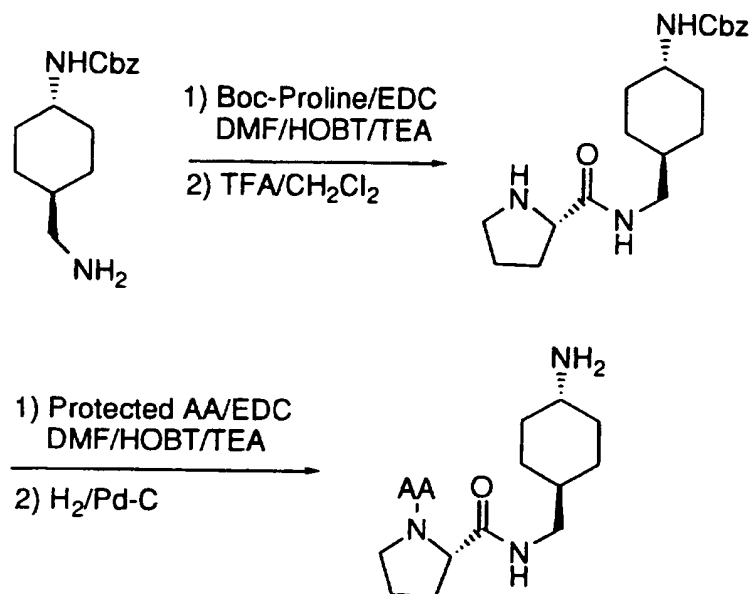
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METHOD 1

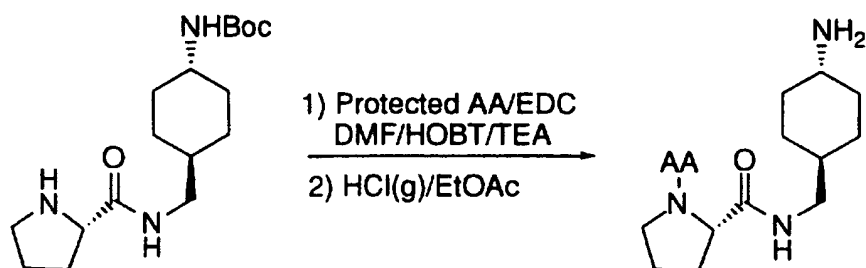
- A second method for constructing the compounds of general structure I (as exemplified by example 18) is to react *trans*-4-benzyloxycarbonylamino-cyclohexyl-methanamine with a protected amino acid such as Boc-L-proline. The Amino acid group is removed and the freed amine is then coupled with the suitable carboxylic acid. The CBZ group is then removed using reducing conditions.
- 5

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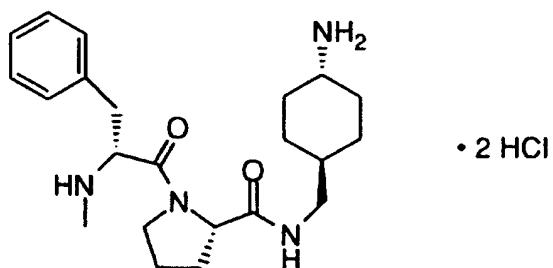
METHOD 2

- 5 A third preferred method (as exemplified by example 5) is to couple the protected Boc-amino-cyclohexylmethanamine-L-proline with the desired acid followed by removal of the Boc protecting group to give the requisite compound.

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METHOD 3

For example, this method can be used to prepare

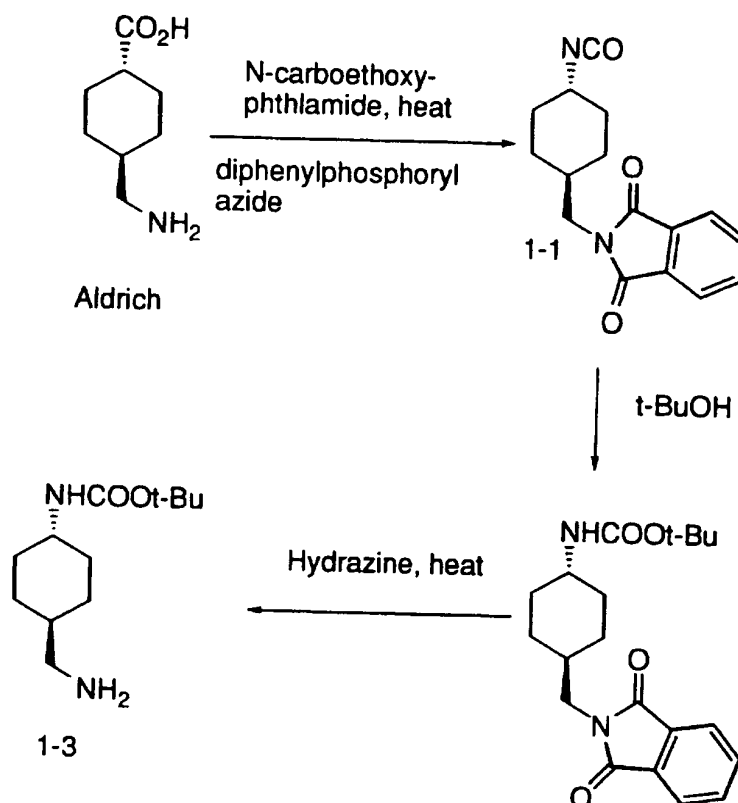


5 as shown in Example 1.

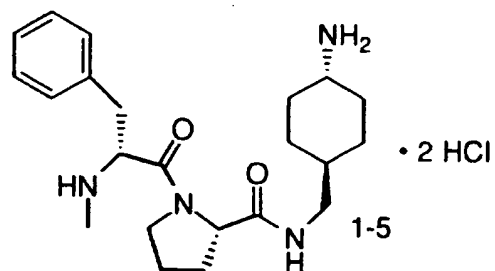
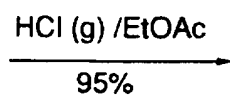
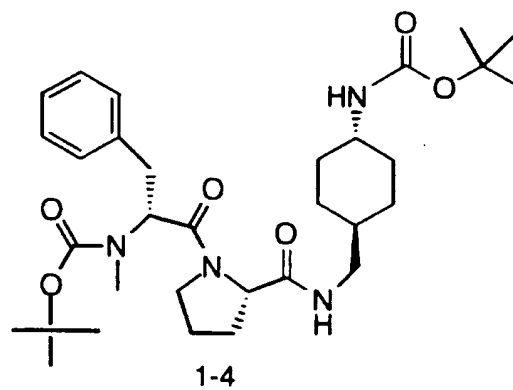
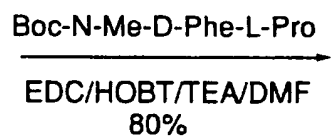
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EXAMPLE 1

Preparation of *trans*-4-Aminocyclohexylmethyl N-Methyl-D-phenylalanyl-L-proline amide



- 30 -



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Step 1: Preparation of N-(*trans*-4-Carboxycyclohexylmethyl)-
phthalimide

N-carboethoxyphthalimide (21.9 g, 0.10 mol), *trans*-4-(aminomethyl)cyclohexane carboxylic acid (15.7 g, 0.10 mol) and triethylamine (14 mL) were stirred in 100 mL THF and the mixture refluxed 18 h. The nearly clear solution was poured into 400 ml water containing 10 mL glac. HOAc with rapid stirring and the precipitated product collected by suction and dried in a vacuum oven at 80°C, mp 190-192°.

Step 2: Preparation of N-(*trans*-4-Isocyanatocyclohexylmethyl)phthalimide

The product from example 1, step 1 was stirred in 200 ml CCl₄ containing 10 mL SOCl₂ and the mixture refluxed under a drying tube until the solution remained clear on cooling and gas evolution ceased. The mixture was concentrated *in vacuo* to 100 ml and treated with 14.0 mL trimethylsilyl azide at reflux for 18 h. The resulting solution was concentrated to give the crude title isocyanate.

Step 3: Preparation of N-(4-tert-butoxycarbonylamino)cyclohexylmethyl phthalimide

The crude product from example 1, step 2 was treated with a solution of lithium tert butoxide in THF for 2 hours at room temperature to give a dark solution which was diluted with aqueous acetic acid and ice to precipitate the crude product which is recrystallized from 1-chlorobutane to give beige needles of the title urethane, mp. 163-165°.

The above urethane phthalimide was treated with 1 equiv anhydrous hydrazine in isopropanol for 18 h at room temperature followed by 4 h reflux. The mixture was concentrated, diluted with cold aqueous acetic acid and filtered to remove phthalazinedione. The aqueous layer was basified with NaOH followed by extraction with ethyl acetate, drying, and evaporation to afford the desired product 1-3 as a solid.

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Step 4: Preparation of *trans*-4-Aminocyclohexylmethyl N-Methyl-D-phenylalanyl-L-proline amide

1-3 (37 mg, 0.16 mmol), Boc-N-Me-D-phenylalanyl-L-proline (60 mg, 0.159 mmol), EDC (31 mg, 0.16 mmol), HOBT•H₂O (22 mg, 0.16 mmol), and triethyl amine (TEA, 0.045 ml, 0.32 mmol) were dissolved in 0.5 ml DMF, adding TEA last. 1.5 ml DMF was added to facilitate stirring. The mixture was stirred under argon overnight.

After overnight stirring, the solution was rotovaped to dryness, partitioned between CHCl₃ and 1M citric acid, washed with 10% Na₂CO₃, dried over MgSO₄ and solvents removed to give 92 mg of a colorless oil.

The oil was dissolved in 3 ml CH₂Cl₂, 1.5 ml TFA was added, and the mixture stirred under argon for 1 h. The stirred solution was rotovaped to dryness, dissolved in CHCl₃, washed with 10% Na₂CO₃, and dried over MgSO₄. Solvents were removed to give 35 mg of an oil.

The oil was chromatographed on 5 g fine SiO₂ using 80:20:2 C-M-NH₄OH. Pure fractions were combined to give 30 mg of colorless glass.

Glass was suspended in 4 ml Et₂O. 17 mL of 9.9 M Ethanolic HCl was added and stirred at room temperature. Filtered off gummy solid, dried at 65°C at high vacuum overnight to give 18 mg of 1-5.

EXAMPLE 2

Preparation of N-*trans*-4-Aminocyclohexylmethyl Hydrocinnamyl-L-proline amide

Step 1: Hydrocinnamyl-L-Proline

Hydrocinnamic acid (1.63 g., 11 mmol) and L-proline-OMe HCl (2.19 g., 13.2 mmol) were dissolved in 110 ml. of dimethylacetamide (DMAc), followed by the addition of 2.19 g. (14.3 mmol) of HOBt and adjustment to pH 8 with N-methylmorpholine

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(NMM). Then EDC (3.16 g., 16.5 mmol) was added and the solution stirred for 20 hr., followed by concentration *in vacuo* and extractive workup with EtOAc to afford 2.97 g. of oily residue, which was purified by chromatography on silica gel to yield 2.68 g. of methyl ester. This intermediate was saponified in 100 ml. of 50:50 THF/H₂O using 5.2 ml. of 2.17 N LiOH for 3 hr., to give, after acidification with dil. KHSO₄, removal of the volatile solvents *in vacuo*, and extractive workup with EtOAc, the title compound as a colorless oil.

10 Step 2: *trans*-4-Aminocyclohexylmethyl Hydrocinnamyl-L-proline amide

Hydrocinnamyl-L-Proline (238 mg., 0.96 mmol) and N-(*trans*-4-tert-butoxycarbonyl)-aminocyclohexylmethylamine (200 mg., 0.88 mmol) were dissolved in 20 ml. of DMF, followed by addition of 15 161 mg. (1.06 mmol) of HOBt and adjustment to pH 8 with NMM. Then EDC (219 mg., 1.14 mmol) was added and the solution stirred for 2 days, followed by concentration *in vacuo* and extractive workup with EtOAc to afford a tacky solid. This material was dissolved in 5 ml. of 100% TFA, let 15 min., and the TFA was removed under reduced pressure and 20 the product purified by preparative HPLC using a TFA(0.1%)-CH₃CN gradient. Lyophilization of pure fractions gave the title compound as a trifluoroacetic acid hydrate salt: C₂₁H₃₁N₃O₂·CF₃COOH·H₂O (FAB MS 358 (M + H⁺)).

25

EXAMPLE 3

Preparation of *trans*-4-Aminocyclohexylmethyl 3-Cyclohexylpropionyl-L-proline amide

A solution of *trans*-4-Aminocyclohexylmethyl hydrocinnamyl-L-proline amide in 50 ml. of 50% HOAc was shaken in a 30 Parr apparatus with 80 mg. of PtO₂ for 20 hr. The solution was decanted and concentrated *in vacuo*, and the residue was dissolved in water and the product purified by preparative HPLC using a TFA(0.1%)-CH₃CN gradient. Lyophilization of pure fractions gave the title compound as a

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trifluoroacetic acid hydrate salt: $C_{24}H_{34}N_4O_4 \cdot CF_3COOH \cdot H_2O$ (FAB MS 443 ($M + H^+$)).

EXAMPLE 4

5

Preparation of *trans*-4-Aminocyclohexylmethyl D-phenylalanyl-L-proline-amide

Boc-D-phenylalanyl-L-proline-OH (293 mg., 0.809 mmol) and N-(*trans*-4-tert-butoxycarbonyl)-aminocyclohexylmethylamine (151 mg., 0.66 mmol) were dissolved in 15 ml. of DMA, followed by addition of 134 mg. (0.876 mmol) of HOBt and adjustment to pH 7 with NMM. Then EDC (167 mg., 0.870 mmol) was added and the solution stirred for 2 days, followed by concentration *in vacuo* and extractive workup with EtOAc to afford 385 mg. of a tacky solid. This material was dissolved in 10 ml. of 100% TFA, let 20 min., and the TFA was removed under reduced pressure and the product purified by preparative HPLC using a TFA(0.1%)-CH₃CN gradient. Lyophilization of pure fractions gave the title compound as a trifluoroacetic acid hydrate salt: $C_{21}H_{32}N_4O_2 \cdot CF_3COOH \cdot H_2O$ (FAB MS 373 ($M + H^+$)).

20

EXAMPLE 5

Preparation of *trans*-4-Aminocyclohexylmethyl 1,4-Benzodioxane-2-carboxy-L-proline amide

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1,4-Benzodioxane-2-carboxylic acid (61 mg., 0.337 mmol) and *trans*-4-(tert-butoxycarbonyl-amino)cyclohexylmethyl L-proline amide (87 mg., 0.266 mmol) were dissolved in 15 ml. of DMAc, followed by addition of 51 mg. (0.330 mmol) of HOBt and adjustment to pH 8 with NMM. Then EDC (63 mg., 0.328 mmol) was added and the solution stirred for 2 days, followed by concentration *in vacuo* and extractive workup with EtOAc to afford 126 mg. of a tacky solid. This material was dissolved in 10 ml. of 1:1 TFA-CH₂Cl₂, let 20 min., and the TFA was removed under reduced pressure and the product purified by preparative HPLC using a TFA(0.1%)-CH₃CN gradient. Lyophilization

30

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of pure fractions gave the title compound as a trifluoroacetic acid hydrate salt: $C_{21}H_{29}N_3O_4 \cdot CF_3COOH \cdot H_2O$ (FAB MS 388 ($M + H^+$)).

EXAMPLE 6

5

Preparation of *trans*-4-Aminocyclohexylmethyl N-Me-D-phenylalanyl-L-azetidine-2-carboxy amide

10 Step 1: Preparation of *trans*-4-Aminocyclohexylmethyl (H) L-Azetidine-2-carboxy amide

Boc-L-azetidine-2-carboxylic acid (91 mg., 0.450 mmol) and N-(*trans*-4-benzyloxycarbonyl-aminocyclohexylmethylamine (89 mg., 0.340 mmol) were dissolved in 15 ml. of DMAc, followed by addition of 77 mg. (0.503 mmol) of HOBt and adjustment to pH 8 with NMM. Then EDC (83 mg., 0.432 mmol) was added and the solution stirred for 2 days, followed by concentration *in vacuo* and extractive workup with EtOAc to afford 140 mg. of a tacky solid. This material was dispersed in 10 ml. of EtOAc, the internal temperature was lowered to -40 deg, and the solution was saturated with HCl (warmed to -10 deg). After 5 min. the mixture was purged with N₂ for 1 hr., and the solvent was removed at reduced pressure to give 115 mg. of the title compound as its HCl salt.

25 Step 2: Preparation of *trans*-4-Aminocyclohexylmethyl N-Me-D-phenylalanyl-L-azetidine-2-carboxy amide

Boc-N-Me-D-phenylalanyl-OH (119 mg., 0.427 mmol) and (H) L-azetidine-2-carboxy-(N-*trans*-4-aminocyclohexylmethyl) amide HCl salt (116 mg., 0.334 mmol) were dissolved in 15 ml. of DMAc, followed by addition of 62 mg. (0.405 mmol) of HOBt and adjustment to pH 8 with NMM. Then EDC (93 mg., 0.484 mmol) was added and the solution stirred for 20 hr., followed by concentration *in vacuo* and extractive workup with EtOAc to afford 179 mg. of a tacky solid. This sample was placed in a Kel-F vessel charged with 0.5 ml. of anisole and 10 ml. of HF, stirred at 0 deg for 1 hr., and the HF was removed under

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reduced pressure. The crude product was precipitated by the addition of 1:1 ether-petroleum ether, collected by filtration, and purified by preparative HPLC using a TFA(0.1%)-CH₃CN gradient. Lyophilization of pure fractions gave the title compound as a trifluoroacetic acid hydrate salt: C₂₁H₃₂N₄O₂·CF₃COOH·H₂O (FAB MS 373 (M + H⁺)).

EXAMPLE 7

Preparation of *trans*-4-Aminocyclohexylmethyl L- and D-(3,3)-diphenylalanyl-L-proline amide

To an ice cooled solution of 250 mg (0.73 mmol) of Boc-D,L-(3,3)-diphenylalanine, 0.204 ml (1.46 mmol) of triethylamine, and 238 mg (0.73 mmol) of *trans*-4-(t butoxycarbonylamino)cyclohexylmethyl proline amide in 2 ml of methylene chloride was added 186 mg (0.73 mmol) of BOP-chloride. The solution was stirred at 0°C for 30 min., then at room temp. for 18 hrs. The reaction was diluted with 3X its' volume of EtOAc and 0.5X its' volume of aq. 10% citric acid solution. The organic layer was washed with water and brine, and was dried (anh. MgSO₄). Filtration and concentration gave a white foam. The crude foam was purified via column chromatography over silica gel with 5% MeOH/CHCl₃ to give 400 mg(85%) of the coupling product as a white foam. The foam was dissolved in 2 ml CH₂Cl₂/2 ml trifluoroacetic acid under a nitrogen atmosphere, and the solution stirred at room temp. for 18 hrs. The reaction was concentrated *in vacuo* to provide a tan foam consisting of a mixture of product diastereomers at the diphenylalanine alpha carbon. The mixture was separated via reverse phase (C₁₈) preparatory HPLC to provide the two pure diastereomers. The more polar diastereomer was isolated as a clear glass. The glass was suspended in Et₂O containing a few drops of EtOAc, and the glass scratched, sonicated, and filtered to give 65 mg of a glassy white solid. Anal. (C₂₇H₂₆N₄O₂ · 2.35 TFA · 0.70 H₂O), CHN. High Res. FABMS: M+1 theo. = 449.29165, obs. = 449.29110. The less polar diastereomer was crystallized in a similar fashion to give 80 mg of a glassy white solid. Anal. (C₂₇H₂₆N₄O₂ · 2.30 TFA · 0.70 H₂O), CHN. High Res. FABMS:

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M+1 theo. = 449.29165, obs. = 449.29128. The less polar diastereomer was the more active isomer in a thrombin inhibition assay, and X-ray crystallography of the thrombin bound complex with this compound indicated that the stereochemistry at the diphenylalanine alpha carbon
5 was (R).

EXAMPLE 8

Preparation of *trans*-4-Amino-cyclohexylmethyl N-(3,3-diphenyl-1-
10 oxo-propan-1-yl)-L-proline amide

To a stirred solution of 100 mg (0.31 mmol) of *trans*-4-(t-Butoxycarbonyl)aminocyclohexylmethyl L-proline amide, 70 mg (0.31 mmol) of 3,3 diphenylpropionic acid, 46 mg (0.34 mmol) of HOBT, and 34 mg (0.34 mmol) of triethylamine in 2 ml of anh. DMF under a
15 nitrogen atmosphere was added 65 mg (0.34 mmol) of EDC. The resulting solution was stirred at room temp. for 18 h. The reaction was diluted with 2X its' volume of aq. 10% citric acid solution, and the mixture extracted 2X with EtOAc. The combined EtOAc extracts were washed with water and brine, and were dried over anh. MgSO₄.

20 Filtration and concentration gave crude product as a yellow oil. The product was purified via column chromatography over silica gel with 3:2 EtOAc/CHCl₃ to give 109 mg (64%) of product as a clear glass.

The product was immediately dissolved in 2 ml CH₂Cl₂/2 ml trifluoroacetic acid, and the solution stirred at room temp. under a
25 nitrogen atmosphere for 18 h. The reaction was concentrated *in vacuo* to give a tan foam. The crude product was purified via reverse phase prep HPLC to provide pure product as a clear glass. The glass was suspended in Et₂O containing a few drops of hexanes, and scratching and filtration provided 65 mg of the desired product as a white solid, MP = 120-123°C.
30 Anal. (C₂₇H₃₅N₃O₂ · 1.60 TFA · 0.45 H₂O), CHN. High Res. FABMS: M+1 theo. = 434.28075, obs. = 434.28204.

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EXAMPLE 9Preparation of *trans*-4-Amino-cyclohexylmethyl N-(benzylsulfonyl)-D and L-3,3-diphenylalanyl-L-proline amide

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Step 1: Preparation of D,L-3,3-diphenylalanine methyl ester hydrochloride

To a stirred solution of 600 mg (1.76 mmol) of Boc-D,L-3,3-diphenylalanine in 7 ml of anh. DMF under a nitrogen atmosphere was added 195 mg (2.32 mmol) of NaHCO₃, followed by 942 mg (6.64 mmol) of CH₃I. The resulting mixture was stirred at room temperature for 18 h. The reaction was diluted with aq. 10% citric acid solution, and the resulting suspension extracted 2X with EtOAc. The combined extracts were washed with water and brine, and were dried over anh. MgSO₄. Filtration and concentration provided a yellow oil. The oil was dissolved in 2 ml of EtOAc, and the solution cooled to 0°C and bubbled with HCL gas for 5 min. The resulting solution was capped and stirred at 0°C for 15 min. The cold solution was scratched, and a white crystalline solid precipitated out. The solid was collected via filtration, washed with Et₂O, and dried *in vacuo* to give 376 mg (73%) of desired product, MP = 221-222 °C (dec.). High Res. FABMS : M+1 theo. = 256.13375, obs. = 256.13287.

Step 2: Preparation of N-(benzylsulfonyl)-D,L-3,3-diphenylalanine-methyl ester

To an ice water cooled solution of 5.80 g (19.88 mmol) of D,L-3,3-diphenylalanine methyl ester hydrochloride and 3.79 g (19.88 mmol) of benzylsulfonyl chloride in 50 ml pyridine in a nitrogen atmosphere was added 2.43 g (19.88 mmol) of 4-dimethylaminopyridine. The suspension was stirred in the bath for 5 min., then at room temp. for 18 h. An additional 0.30 eq. of each reagent was added, and stirring continued for an additional 4 h. The reaction was concentrated *in vacuo*, and the residue partitioned between aq. 10% citric acid solution and EtOAc. The aqueous layer was reextracted with EtOAc, and the

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combined extracts washed with water and brine. Drying and concentration *in vacuo* provided 6g of crude product as a tacky yellow solid. Column chromatography over silica gel with 14:1 CHCl₃/EtOAc provided 4.85g(60%) of desired product as a white cryst. solid, MP = 162-163°C. High Res. FAB MS: M⁺ theo. = 410.14260, obs. = 410.14303.

Step 3: Preparation of *trans*-4-Amino-cyclohexylmethyl N-(benzylsulfonyl)-D and L-3,3-diphenylalanyl-L-proline amide

A solution of 215 mg (0.53 mmol) of N-(benzylsulfonyl)-D,L-3,3-diphenylalanine-methyl ester in 3 ml aq. 2N LiOH/3 ml DME was stirred at room temperature for 48 h. The reaction was conc. to remove DME, and was acidified with aq. 10% citric acid solution. The aqueous soln. was extracted 2X with EtOAc. The combined extracts were washed with water and brine and were dried (anh. MgSO₄). Filtration and concentration provided 200 mg (95%) of the free proline acid. A stirred solution of the 200 mg (0.51 mmol) sample of acid, 166 mg (0.51 mmol) of *trans*-4-(t-butoxycarbonyl)amino-cyclohexylmethyl L-proline amide, and 184 mg (0.51 mmol) of chloro-N,N,N',N'-bis(pentamethylene) formamidine hexafluorophosphate (Fluka) in 2 ml CH₂Cl₂ cooled to 0°C in a nitrogen atmosphere was treated with 132 mg (1.02 mmol) of diisopropylethylamine. The solution was stirred at 0°C for 5 min., and was stirred at room temp. for 2 h. The solution was concentrated *in vacuo*, and the residue partitioned between aq. 10% citric acid and EtOAc. The aqueous layer was reextracted 2X with EtOAc, and the combined extracts washed with 5% aq. NaHCO₃ soln., water, and brine. Drying (anh. MgSO₄) and concentration *in vacuo* provided crude product as a tan foam. The foam was purified via column chromatography over silica gel with 5%MeOH/CHCl₃ to give 161 mg (44% based on acid) of the coupling product. The product was dissolved in 1.5 ml CH₂Cl₂/1.5 ml trifluoroacetic acid, and the solution stirred at room temp. in a nitrogen atmosphere for 2 h. The reaction was concentrated *in vacuo* to provide a yellow oil, which consisted of a

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mixture of diastereomers at the diphenylalanine alpha carbon. The mixture was separated via reverse phase (C18) preparatory HPLC to provide pure samples of each diastereomer. The earlier eluting diastereomer was isolated after lyophilization as a clear glass. The glass was suspended in ether, scratched and filtered to give 30 mg of pure more polar diastereomer as a white glassy solid. Anal. (C₃₄H₄₂N₄O₄S · 1.35 TFA · 2.00 H₂O), CHN. FAB MS : M+1 = 603. The less polar diastereomer was isolated after lyophilization as a white glassy powder. Anal. (C₃₄H₄₂N₄O₄S · 1.45 TFA · 0.70 H₂O), CHN. High Res. FAB MS: M+1 theo. = 603.30050, obs. = 603.29885. The less polar diastereomer was tentatively assigned the (R) stereochemistry (corresponds to the (D) amino acid) at the diphenylalanine alpha carbon, as it was determined to be the more active isomer in a thrombin inhibition assay.

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EXAMPLE 10

Preparation of *trans*-4-Aminocyclohexylmethyl N-(2-naphthylsulfonyl)-glycyl-L-proline amide.

To a stirred solution of 82 mg (0.34 mmol) of N-(2-naphthylsulfonyl)glycine, 100 mg (0.31 mmol) of *trans*-4-(t-butoxycarbonyl)aminocyclohexylmethyl L-proline-amide, 46 mg (0.34 mmol) HOBT, and 34 mg (0.34 mmol) of triethylamine in 2 ml anh. DMF under a nitrogen atmosphere was added 65 mg (0.34 mmol) of EDC, and the solution stirred at room temperature for 18 h. The reaction was diluted with 2X its volume of aq. 10% citric acid solution, and was extracted 2X with EtOAc. The combined EtOAc extracts were washed with water and brine, and dried over anh. MgSO₄. Concentration provided approx. 200 mg of a white foam. The crude product was purified via column chromatography over silica gel with 4% MeOH/CHCl₃ to give 90 mg of purified coupling product as a clear glass. The glass was dissolved in 2 ml EtOAc/0.5 ml CHCl₃, and the solution cooled to 0°C. The solution was bubbled with HCl gas for 5 min. at 0°C, and was stirred in the cold for an additional 20 min.

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- Scratching the side of the flask with a glass rod induced crystallization, and the precipitated product was filtered off and washed with EtOAc and Et₂O. Drying provided 50 mg of product as a white solid, MP = 189-191°C. Anal.(C₂₄H₃₂N₄O₄S · HCl·0.20 CHCl₃ · 0.20 H₂O), CHN.
- 5 FAB MS: M=1 = 473.

EXAMPLE 11

- Preparation of *trans*-4-Aminocyclohexylmethyl N-(benzylsulfonyl)-D-phenylalanyl-L-proline amide
-

- Step 1: Preparation of N-benzenesulfonyl-L-phenylalanine
(D)-PheOH (0.83g 5.0 mmol) was dissolved in 40 mL dioxane by addition of 5 ml 1N NaOH. The resulting solution was
- 15 treated dropwise with phenylmethanesulfonyl chloride with rapid stirring at room temperature. After 1 hour, the mixture was diluted with 10 mL H₂O and filtered. The filtrate was treated with 2.5 mL aq. KHSO₄ and concentrated in vacuo. Extraction of the residue with CHCl₃ and concentration of the CHCl₃ gave the title compound which melted at
- 20 150°-152°C (n-butylchloride).

- Step 2: Preparation of *trans*-4-Aminocyclohexylmethyl N-phenylmethanesulfonyl-D-phenylalanyl-L-proline amide
N-phenylmethanesulfonyl-D-phenylalanine (108 mg, 0.33
- 25 mmol) and N-(*trans*-4-tert. butyloxycarbonylamino)cyclohexylmethyl L-proline amide (130 mg) were coupled with hydroxybenztriazole hydrate (55 mg) and EDC-HCl (70 mg) in 1 mL DMF containing triethylamine (100 ml). The mixture was stirred under N₂ at room temperature overnight, then diluted with 10 mL of 10% aqueous citric acid and
- 30 extracted with CH₂Cl₂. The CH₂Cl₂ extracts were washed with aqueous Na₂CO₃, dried (Na₂SO₄), filtered and concentrated in vacuo to give the crude Boc derivative of the title compound. Chromatography on silica gel afforded the pure Boc derivative (130 mg) which was treated with 0.3 mL trifluoroacetic acid in 1 mL CH₂Cl₂ for 6 hours at room temperature.

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The mixture was concentrated and the residue purified by preparative HPLC using a trifluoroacetic acid (0.1%)-CH₃CN gradient.

Lyophilization of pure fractions gave the title compound as a trifluoroacetic acid hydrate salt: C₂₈H₃₈N₄O₄S•CF₃CO₂H•H₂O (FAB

5 MS 527.1 (M+ H⁺)) L-372,130.

EXAMPLE 12

10 Preparation of *trans*-4-aminocyclohexylmethyl N-methanesulfonyl-D-phenylalanyl-L-proline amide

Step 1: Preparation of N-methanesulfonyl-D-phenylalanyl

H-D-phenylalanyl-benzylester (toluenesulfonate salt, 2.14 g, 5.0 mmol) and triethylamine (1.4 mL) were dissolved in 25 mL CH₂Cl₂ and the solution treated with methanesulfonyl chloride (0.39 mL, 5.0 mmol). After 30 minutes at room temperature, the solution was washed with 1.2 N HCl, water, and dried (Na₂SO₄). Concentration of the filtered CH₂Cl₂ gave 2.3 g of the benzyl ester. The crude product was crystallized from n-butyl chloride to give a white solid, mp 76°-77°.

20 The crystalline ester was hydrogenolyzed in 50 mL abs. EtOH with 100 mg 10% Pd-C and balloon pressure of H₂. After 18 hours at room temperature, the mixture was filtered through celite and the filtrate concentrated. The crude title acid was crystallized by trituration with n-butyl chloride, mp 103°-105°.

25

Step 2: Preparation of *trans*-4-Aminocyclohexylmethyl N-methanesulfonyl-D-phenylalanyl-L-proline amide

The acid of Example 13, step 1 was coupled with N-(*trans*-4-tert. butyloxycarbonylamino-cyclohexylmethyl) L-proline amide using standard conditions. The crude Boc protected intermediate was deblocked with CF₃CO₂H in CH₂Cl₂ and the crude product crystallized by trituration with ether to give the title compound C₂₂H₃₄N₄O₄S•CF₃CO₂H•H₂O).

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EXAMPLE 13

Preparation of *trans*-4-Aminocyclohexylmethyl *trans*-threo-5-phenyl-oxazolidine-4-carbonyl-L-proline amide

- 5 dl-*trans*-threo-5-Phenyl-oxazolidine-4-carboxylic acid (21 mg, 1.05 mmol) was coupled with *trans*-4-(tert. butoxycarbonyl)aminocyclohexylmethyl L-proline amide (330 mg, 1.0 mmol) using hydroxybenztriazole hydrate (150 mg) and EDC. HCl (200 mg) with triethylamine in DMF as in previous examples. The crude
- 10 reaction product from CH₂Cl₂ extraction was crystallized from EtOAc to give a solid fraction rich in one diastereomer (140 mg). This solid was deblocked with TFA in CH₂Cl₂ and the crude product purified by preparative HPLC (CF₃CO₂H-H₂O:CH₃CN) to give diastereomer A of the title compound after trituration with EtOAc-Et₂O (CN, CH for
- 15 C₂₂H₃₀N₄O₆•CF₃CO₂H•1.65 H₂O); HPLC 99% pure A, 4-(R).

The EtOAc soluble portion of the coupling product was deblocked with CF₃CO₂H in CH₂Cl₂ and the diastereomer B purified by preparative HPLC (CF₃CO₂H-H₂O:CH₃CN) (N, C, H for C₂₂H₃₀N₄O₂•CF₃CO₂H•1.65 H₂O, FAB MS 415 (M+H⁺)); 4-(S).

20

EXAMPLE 14

Preparation of *trans*-4-(benzyloxycarbonyl)aminocyclohexylmethyl amine

- 25 The crude isocyanate from example 1, step 3 was treated with 1 equiv. benzyl alcohol and triethylamine in THF at reflux for 18 h under N₂. The product slowly crystallizes and is recovered by concentrating the cooled reaction mixture, diluting with ice cold aqueous acetic acid and suction filtration. Recrystallization from CH₂Cl₂-hexane
- 30 gives the title urethane, mp 139-140°.

The above urethane phthalimide was treated with 1 equiv anhydrous hydrazine in isopropanol for 18 h at room temperature followed by 4 h reflux. The mixture was concentrated, diluted with cold aqueous acetic acid and filtered to remove phthalazinedione. The

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aqueous layer was basified with NaOH followed by extraction with ethyl acetate, drying, and evaporation to afford the desired product as a solid. mp 118-121

5

EXAMPLE 15

Preparation of *trans*-4-Amino-cyclohexylmethyl N -(3,3-dicyclohexyl-1-oxo-propan-1-yl)-L-proline amide

In a manner similar to that used in example 9, but
10 substituting 3,3-dicyclohexylpropionic acid for 3,3-diphenylpropionic acid, the protected dicyclohexyl analog was prepared. Removal of the BOC group with HCl gas in cold EtOAc followed by trituration with Et₂O and filtration provided the desired product as a white solid, MP = 104-107°C. High Res. FAB MS: M⁺ theo. = 446.37465, obs. =
15 446.37430. Anal. (C₂₇H₄₇N₃O₂ · HCl · 0.35 H₂O) C,H,N.

EXAMPLE 16

Preparation of *trans*-4-Amino-cyclohexyl]-methyl N -3-(R) or (S)
20 phenyl-3-cyclohexyl-1-oxo-propan-1-yl)-L-proline amide

In a manner similar to that described in example 16, but substituting 3-(R,S)-cyclohexyl-3-(R,S)-phenyl-propionic acid for 3,3-dicyclohexylpropionic acid, the 3-cyclohexyl-3-phenyl compound was prepared as a mixture of diastereomers. The diastereomers were
25 separated via reverse phase prep LC to provide after lyophilization each diastereomer as a white fluffy solid.

More polar diastereomer MP = 116-119°C, FAB MS : M⁺ = 440; contains approx. 7% of the other diastereomer by 400 MHz NMR.
Anal.(C₂₇H₄₁N₃O₂ · 1.25 TFA · 0.80 H₂O) C,H,N.

30 Less polar diastereomer MP = 120 -122°C, FAB MS: M⁺ = 440; contains approx. 4% of the other diastereomer by 400 MHz NMR.
Anal.(C₂₇H₄₁N₃O₂ · 1.25 TFA · 0.65 H₂O) C,H,N.

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EXAMPLE 17Preparation of *trans*-4-Aminocyclohexylmethyl L- and D-3,3-Dicyclohexylalanyl-L-proline amide

5

Step 1: Preparation of N-CBZ-D,L-3,3-dicyclohexylalanine

A solution of 2.00g of D,L-3,3-diphenylalanine HCl in 50 ml acetic acid/10 ml H₂O was hydrogenated at 62 psi on a Parr apparatus over 500 mg of Ir black catalyst. After 24h, a second portion of catalyst was added and the reaction continued for a second 24 h interval. The reaction was filtered through a Celite pad, and the filtrate concentrated *in vacuo* to give a tan foam. The foam was diluted with Et₂O, scraped, and sonicated to give 1.38g of D,L-3,3-dicyclohexylalanine HCl as a tan solid, MP = 261-264°C. The amino acid (4.76 mmol) was dissolved in 40 ml of 2N NaOH, and the solution cooled to 0°C. The solution was treated dropwise with 1.06 g(6.19 mmol) of benzyl chloroformate with the temp. maintained at < 5°C. After completion of the addition, the reaction was stirred at 0°C for 15 min., then at room temp. for 1h. The suspension was acidified to pH 2 with 2.75 M KHSO₄ solution, and the suspension extracted with 3 x 50 ml of EtOAc. The combined extracts were dried, decolorized with activated carbon, and filtered through a Celite pad. Concentration provided a peach colored oil which partially crystallized on pumping. The residue was triturated with hexanes which induced further crystallization. Filtration provided 1.00 g (55%) of desired product as a white crystalline solid, MP = 150 - 152 °C. High Res. FABMS: M+ theo. = 388.24878 ; obs. = 388.24793.

Step 2: Preparation of N-CBZ-L- and D-3,3-Dicyclohexylalanyl-L-proline

30

A solution of 850 mg (2.19 mmol) of N-CBZ-D,L-3,3-dicyclohexylalanine, 363 mg (2.19 mmol) of proline methyl ester HCl, 325 mg (2.41 mmol) of EDC, and 488 mg (4.82 mmol) of triethylamine in 12 ml of anh. DMF was treated with 462 mg (2.41 mmol) of EDC, and the resulting solution stirred at room temp. in an N₂ atmosphere for 18h.

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The reaction was diluted with 3x its volume of 10% citric acid solution, and the suspension extracted with 2 x 40 ml of EtOAc. The combined EtOAc extracts were washed with water and brine, and were dried and concentrated to provide the crude coupling product. The crude product was purified via column chromatography over silica gel with 2.5 % MeOH/CHCl₃ to give the pure coupling product as a white foam. The foam was dissolved in 5 ml 2M LiOH/ 5 ml DME, and the solution stirred vigorously at room temp. for 18 h. The reaction was acidified with 10% citric acid solution and extracted with EtOAc. The extract was washed with brine, dried, and concentrated to provide the crude N-CBZ-L- and D-3,3-dicyclohexylalanine-L-proline as a mixture of diastereomers. The acid diastereomers were separated via reversed phase preparatory HPLC to provide 370 mg of the more polar diastereomer as a white foam. High Res. FABMS: M+ theo. = 485.30154, obs. = 485.30090. 363 mg of the less polar diastereomer was also obtained as a glass. High Res. FABMS: M+ theo. = 485.30154, obs. = 485.30162. Each diastereomer was completely free of the other diastereomer by analytical HPLC and NMR.

20 Step 3: Preparation of L- and D-3,3-Dicyclohexylalanyl-L-proline-N-[*trans*-4-aminocyclohexyl]methyl amide

A solution of 361 mg (0.75 mmol) of the more polar diastereomer of N-CBZ-3,3-Dicyclohexylalanyl-L-proline, 197 mg (0.75 mmol) of *trans*-(4- N-CBZ-aminocyclohexyl)methyl amine, 112 mg (0.83 mmol) of HOBT, and 84 mg (0.83 mmol) of triethylamine in 5 ml anh. DMF was treated with 159 mg (0.83 mmol) of EDC, and the resulting solution stirred at room temp. in an N₂ atmosphere for 18 h. The reaction was diluted with 3X its volume of water, and the suspension stirred vigorously at room temp. for 15 min. The suspension was filtered, and the white solid washed with water and dried. 528 mg (97%) of the bis-CBZ protected coupling product was obtained, MP = 79-82°C. A 500 mg sample of this material was dissolved in 40 ml of 4:1 EtOH/water, and was hydrogenated on a Parr apparatus at 50 psi over 150 mg of Pd(OH)₂ catalyst for 18h. The reaction was filtered through a

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Celite pad, and the filtrate concentrated to provide the crude product as a clear oil. The oil was purified via reverse phase prep LC to provide 330 mg of the desired product after lyophilization as an amorphous glass. HR FABMS: M+ theo. = 461.38555, obs. = 461.38646. Anal. (C₂₇H₄₈N₄O₂ · 2.40 TFA · 0.45 H₂O) C, H, N.

An identical procedure performed on 348 mg (0.72 mmol) of the more polar diastereomer of N-CBZ-Dicyclohexylalanine-L-proline provided 275 mg of product as an amorphous tacky solid. HR FABMS: M+ theo. = 461.38646, obs. = 361.38664. Anal. (C₂₇H₄₈N₄O₂ · 2.50 TFA · 0.50 H₂O) C, H, N. This material was the more active diastereomer in a Thrombin inhibition assay, and was therefore assigned the (R)-configuration at the dicyclohexylalanine alpha position.

EXAMPLE 18

15

Preparation of *trans*-4-Aminocyclohexylmethyl N-BOC-L- and D-3,3-Dicyclohexylalanyl-L-proline amide

Step 1: Preparation of N-BOC-D,L-3,3-dicyclohexylalanine

To a solution of 2.00g (6.90 mmol) of D,L-3,3-dicyclohexylalanine in 17 ml 1N NaOH/9 ml water/17 ml 1,4 dioxane cooled to 0°C was added 1.66 g (7.59 mmol) of di-(t-butyl) dicarbonate in portions over approx. 2 min. The solution was stirred in the cold for 5 min., then at room temp. for an additional 2h. The reaction was concentrated to remove most of the dioxane, and was cooled in an ice bath. The cold reaction was acidified to pH 2 with 1N KHSO₄, and was extracted 2x with EtOAc. The combined extracts were washed with water and brine, and were dried and concentrated to provide 2.43 g(90%) of the desired product as a white crystalline solid. MP = 188.0-189.5 °C.

High. Res. FABMS:
M+ theo. = 354.26443, obs. = 354.26588.

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Step 2: Preparation of N-BOC-L- and D-3,3-Dicyclohexylalanyl-L-proline

In a manner identical to the example 18, step 2, from 2.10 g (5.94 mmol) of N-BOC-D,L-3,3-dicyclohexylalanine was obtained 0.82 g of the more polar acid diastereomer as a clear oil/foam, HR FABMS M+ theo. = 451.31720, obs. = 451.31764. A 1.20 g quantity of the less polar diastereomer was also obtained, HR FABMS M+ theo. = 451.31720, obs. = 451.31587. Each diastereomer was free of any of the other diastereomer by analytical HPLC and NMR.

Step 3: Preparation of trans-4-Aminocyclohexylmethyl N-BOC-L- and D-3,3-Dicyclohexylalanyl-L-proline amide

In a manner identical to example 18, step 3, from 264 mg of the more polar diastereomer of N-BOC-3,3-Dicyclohexylalanyl-L-proline was obtained 135 mg of the desired product as a clear glass. FAB MS : M+ = 561.

Also in an identical manner from 370 mg of the less polar diastereomer of N-BOC-3,3-Dicyclohexylalanyl-L-proline was obtained 155 mg of desired product as a clear glass. FAB MS: M+ = 561. Anal.(C₃₂H₅₆N₄O₄ . 0.70 EtOAc . 0.15 H₂O) C, H, N.

EXAMPLE 19

Preparation of trans-4-t-butoxycarbonylaminocyclohexylmethyl L-proline amide

Step 1: Preparation of trans-4-allyloxycarbonylaminomethyl-cyclohexanecarboxylic acid

A stirred solution of 34.2 g (217.5 mmol) of trans 4-aminomethylcyclohexanecarboxylic acid (Aldrich) in 217 mL of 2M aq. NaOH and 217 mL of dioxane was cooled to 15°C. To this solution was added 25 mL (235 mmol) of allyl chloroformate (Aldrich) in a slow stream. After the addition, the cold bath was removed, and the solution was stirred overnight at ambient temperature. The reaction mixture was

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poured slowly into a rapidly stirred mixture of 1L of 1M aq. citric acid and ice. The resulting mixture was stirred for 0.5 h and the precipitate collected by filtration, washed with water, and dried to a constant weight of 49.3 g (94%) of the title compound as a colorless solid.

5

Step 2: Preparation of *trans*-4-t-butoxycarbonylamino-cyclohexyl-(allyloxycarbonyl)methylamine

A stirred solution of 15.0 g (62.2 mmol) of *trans*-4-allyloxycarbonylamino-methylcyclohexanecarboxylic acid in 550 mL of benzene and 9.1 mL (65.3 mmol) of triethylamine was heated to 45°C under Ar. Diphenylphosphoryl azide (14.1 mL, 65.3 mmol, Aldrich) was added in one portion, and the solution stirred for 24 h at 45 °C. The solution was cooled to 10°C and 65 mL of a 1.0 M solution of lithium t-butoxide in THF (Aldrich) was added such that the reaction temperature remained between 8-10°C. A second 65 mL portion of t-butoxide solution was added, and the solution stirred for 45°C at 8-12°C. This solution was then poured into a rapidly stirred mixture of 1M aq. citric acid and ice, and the resulting mixture stirred for 1 h. This mixture was extracted with 2 portions of ethyl acetate, the combined organic layers washed with 2 x 10% Na₂CO₃, brine, and dried over Na₂SO₄. This solution was poured through a pad of 150 g of silica gel, and the pad washed with 2 x 300 mL of ethyl acetate. Evaporation of the filtrate gave 15.9 g (82%) of the title compound as a solid.

25 Step 3: Preparation of *trans*-4-t-butoxycarbonylamino-cyclohexyl-methylamine

To an Ar filled flask containing 5 g (4.33 mmol) of tetrakis(triphenylphosphine) palladium (Aldrich) and 15.92 g (50.96 mmol) of *trans*-4-t-butoxycarbonylamino-cyclohexyl-(allyloxycarbonyl)methylamine was added 220 mL of dry THF. After the solids had dissolved, the solution was stirred for 5 min. and 50 mL (483 mmol) of diethylamine (Aldrich) was added in one portion. The solution was stirred at ambient temperature overnight. The solvents were removed by rotovap, and the residue partitioned between ice-cold 0.5 M aq HCl and

30

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ethyl acetate. The organic layer was extracted with cold 0.5 M aq HCl, and the combined aqueous layers washed with ethyl acetate, basified with aq NaOH and extracted with 3 x ethyl acetate. The combined organic layers were washed with water, brine, dried over Na₂SO₄ and solvents removed to give an oil that was chromatographed on 250 g of fine SiO₂ using 90:10:1 chloroform-methanol-ammonium hydroxide to give 7.9 g (56%) of the title compound as a colorless semi-solid: NMR (CDCl₃) δ 0.5-1.5 (m, 4H), 1.8-1.3 (m, 1H), 1.44 (s, 9H), 1.81 (dm, J = 10.3, 2H), 2.03 (dm, J = 10.3, 2H), 2.53 (d, J = 6.6, 2H), 3.37 (br s, 1H), 4.44 (br s, 1H).

Step 4: Preparation of *trans*-4-t-butoxycarbonylaminocyclohexylmethyl L-proline amide

To a stirred solution of 1.70 g (6.8 mmol) of Cbz-L-proline (Bachem), 1.54 g (6.74 mmol) of *trans*-4-t-butoxycarbonylaminocyclohexylmethylamine, and 919 mg (6.8 mmol) of 1-hydroxybenzotriazole hydrate (Aldrich) in 11 mL of DMF under Ar was added 1.30 g (6.8 mmol) of EDC and 1.90 mL (13.6 mmol) of triethylamine. The solution was stirred at ambient temperature overnight. The solvents were removed by rotovap and the residue partitioned between CHCl₃ and 1M aq citric acid. the aqueous layer was extracted with CHCl₃ and the combined organic layers were washed with 10% Na₂CO₃, dried over MgSO₄ and the solvents removed to give 3.0 g of a glassy foam. A flask containing a solution of this material in 150 mL of absolute ethanol and 1.5 mL of acetic acid was treated with 1 g of 10% palladium on carbon and fitted with a balloon filled with hydrogen. After stirring for 7.5 h, the mixture was degassed and filtered to give a solution that was concentrated by rotovap. The resulting residue was partitioned between CHCl₃ and cold 1M aq NaOH. The organic layer was washed with water, dried over Na₂SO₄ and solvents removed to give a semi-solid that was chromatographed on 100 g fine SiO₂ using 93:7:0.7 chloroform-methanol-ammonium hydroxide to afford 1.05 g of the title compound as a colorless solid: NMR (CDCl₃) δ 0.96-1.14 (m, 4H), 1.44 (s, 9H), 1.66-1.82 (m, 4H), 1.83-2.06 (m, 5H), 2.06-2.19 (m, 1H), 2.83-2.93 (m,

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1H), 2.93-3.15 (m, 3H), 3.38 (br s, 1H), 3.72 (dd, J = 9.1, 5.3, 1H), 4.01 (br s, 1H), 7.73 (br s, 1H).

EXAMPLE 21

5

Preparation of *trans*-4-Aminocyclohexylmethyl N-2-cyclohexylethyl-3-cyclohexyl-D-alanyl-L-proline amide dihydrochloride salt

10 Step 1: Preparation of *trans*-4-Aminocyclohexylmethyl N-2-phenylethyl-3-cyclohexyl-D-alanyl-L-proline amide

To a stirred mixture of 241 mg (0.503 mmol) of *trans*-4-aminocyclohexylmethyl 3-cyclohexyl-D-alanyl-L-proline amide, 65 μ L (0.554 mmol) of phenylacetaldehyde (Aldrich), and 102 mg (3.78 mmol) of aluminum foil cut in small pieces in 13 mL of methanol was added 15 13.7 mg (0.05 mmol) of mercuric chloride. The mixture was stirred overnight under Ar. Filtered through a pad of Celite and washed with methanol. The filtrate was evaporated by rotovap, the residue dissolved in ethyl acetate, washed with 2 x sodium potassium tartrate, brine, dried over Na₂SO₄ and the solvents removed to give a residue which was 20 chromatographed on SiO₂ using 96:4 chloroform-methanol to give 260 mg of an oil. This oil was dissolved in 40 mL of ethyl acetate, cooled to 0°C and saturated with HCl gas. The flask was stoppered and stirred in the cold for 0.5 h. The solution was sparged with a stream of Ar for 1 h and concentrated to give an oil which was chromatographed on SiO₂ 25 using 99:1 chloroform-methanolic ammonia to give 120 mg of the title compound as a colorless solid.

EXAMPLE 22

30 Preparation of *trans*-4-Aminocyclohexylmethyl N-2-cyclohexylethyl-3-cyclohexyl-D-alanyl-L-proline amide

A solution of 85 mg (0.176 mmol) of *trans*-4-Aminocyclohexylmethyl N-2-phenylethyl-3-cyclohexyl-D-alanyl-L-proline amide in 10 mL of ethanol and 3 mL of acetic acid was

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hydrogenated at 50 psi in the presence of 33 mg of platinum oxide overnight. The catalyst was removed by filtration and washed with ethanol. Concentration of the filtrate afforded a residue which was partitioned between EtOAc and 1M aq. NaOH. The organic layer was
5 washed with water, brine, dried over Na₂SO₄ and the solvents removed to give 87 mg of an oil. This material was dissolved in ethyl acetate and treated with ethanolic HCl to afford the title compound as a colorless dihydrochloride salt.

10

EXAMPLE 23

Preparation of *trans*-4-Aminocyclohexylmethyl N-[3-(5H-dibenzo[a,d]-cycloheptene-5-yl)-1-oxopropan-1-yl]-L-proline amide

To a stirred solution of 87.60 mg(0.35 mmol) of 5H-
15 dibenzo[a,d]-cycloheptene-5-acetic acid, 125.00 mg(0.35 mmol) of *trans*-

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EXAMPLE 24

Preparation of *trans*-4-Aminocyclohexylmethyl N-[3-(10,11-dihydro-5H-dibenzo[a,d]-cycloheptene-5-yl)-1-oxopropan-1-yl]-L-proline amide

- 5 To a stirred solution of 129 mg (0.52 mmol) of 10,11-dihydro-5H-dibenzo[a,d]-cycloheptene-5-acetic acid, 180 mg (0.52 mmol) of *trans*-4-aminocyclohexylmethyl L-proline amide, 76 mg (0.56 mmol) HOBt, and 57 mg (0.56 mmol) of TEA in 3 ml of anh. DMF under an N₂ atmosphere was added 107 mg (0.56 mmol) of EDC. The
- 10 resulting solution was stirred at room temp. for 18 h. The reaction was diluted with 3X its' volume of water, and the suspension stirred vigorously at room temp. for 20 min. Filtration and drying in vacuo provided an off white solid which became quite tacky. The tacky oil/solid was dissolved in EtOAc, dried, filtered, and the filtrate conc. to
- 15 give 138 mg of coupling product as a white foam.

- The coupling product was dissolved in 8 ml of EtOAc, and the solution was cooled to 0°C. The cold solution was bubbled with HCl gas for 3 min., capped, and stirred in the cold for approx. 30 min. The cold rxn. was purged with N₂, and a tacky precipitate formed. The
- 20 material was filtered off, but became extremely tacky despite numerous crystallization attempts. The material was dissolved in EtOAc, dried, filtered and reisolated as a crude foam. Reverse phase prep LC provided 75 mg of the pure product as an amorphous white solid after lyophilization. Anal.(C₂₉H₃₇H₃O₂ · 1.60 TFA · 0.20 H₂O), CHN. High
- 25 Res. FAB MS: M+1 theo. = 460.29640, obs. = 460.29665.

EXAMPLE 25

- Preparation of *trans*-4-Aminocyclohexylmethyl 9-hydroxyfluorene-9-carboxy-L-proline amide

9-Hydroxyfluorene-9-carboxylic acid (251 mg., 1.11 mmole) and *trans*-4-(tert-butoxycarbonyl-amino)cyclohexylmethyl L-proline amide (300 mg., 0.266 mmol) were dissolved in 15 ml of dimethyl acetamide, followed by addition of 170 mg. (1.11 mmole) of

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HOBT and 256 mg. (1.33 mmole) of EDC, and adjustment to pH 8 with NMM. The reaction was monitored to completion via TLC, then concentrated *in vacuo*, and extractive workup with EtOAc yielded 495 mg of crude Boc protected material. This product was dissolved in 10 ml. of 1:1 TFA-CH₂Cl₂, let 20 min., and the TFA was removed under reduced pressure and the product purified by preparative HPLC using a TFA(0.1%)-CH₃CN gradient. Lyophilization of pure fractions gave the title compound as a trifluoroacetic acid hydrate salt:
C₂₆H₃₁N₃O₃·CF₃COOH·H₂O (FAB MS 434 (M + H) and 416 (M + H - H₂O)).

EXAMPLE 1

Tablet Preparation

15

Tablets containing 25.0, 50.0, and 100.0 mg., respectively, of the following active compounds are prepared as illustrated below:

20 *trans*-4-Amino-cyclohexylmethyl N-(3,3-diphenyl-1-oxo-propan-1-yl)-L-proline amide;

trans-4-Aminocyclohexylmethyl N-[3-(5H-dibenzo[a,d]-cycloheptene-5-yl)-1-oxopropan-1-yl]-L-proline amide; and

25 *trans*-4-t-butoxycarbonylaminocyclohexylmethyl L-proline amide.

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TABLE FOR DOSES CONTAINING FROM
25-100MG OF THE ACTIVE COMPOUND

		<u>Amount-mg</u>	
		25.0	50.0 100.0
Active Compound	25.0	50.0	100.0
Microcrystalline cellulose	37.25	100.0	200.0
Modified food corn starch	37.25	4.25	8.5
Magnesium stearate	0.50	0.75	1.5

- 5 All of the active compound, cellulose, and a portion of the corn starch are mixed and granulated to 10% corn starch paste. The resulting granulation is sieved, dried and blended with the remainder of the corn starch and the magnesium stearate. The resulting granulation is then compressed into tablets containing 25.0, 50.0, and 100.0 mg, respectively, of active ingredient per tablet.
- 10

EXAMPLE 8

- 15 An intravenous dosage form of the above-indicated active compound is prepared as follows:

Active Compound	0.5-10.0mg
Sodium Citrate	5-50mg
Citric Acid	1-15mg
Sodium Chloride	1-8mg
Water for Injection (USP)	q.s. to 1 ml

- 20 Utilizing the above quantities, the active compound is dissolved at room temperature in a previously prepared solution of sodium chloride, citric acid, and sodium citrate in Water for Injection (USP, see page 1636 of United States Pharmacopeia/National Formulary for 1995, published by United States Pharmacopeial Convention, Inc., Rockville, Maryland, copyright 1994.

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In Vitro Assay For Determining Proteinase Inhibition

Assays of human α -thrombin and bovine trypsin were performed at 25°C in 0.05 M TRIS buffer pH 7.4, 0.15 M NaCl, 0.1% PEG. Trypsin assays also contained 1 mM CaCl_2 . In assays wherein rates of hydrolysis of a *p*-nitroanilide (pna) substrate were determined, a Thermomax 96-well plate reader was used to measure (at 405 nm) the time dependent appearance of *p*-nitroaniline. sar-PR-pna was used to assay human α -thrombin ($K_m=125 \mu\text{M}$) and bovine trypsin ($K_m=125 \mu\text{M}$). *p*-Nitroanilide substrate concentration was determined from measurements of absorbance at 342 nm using an extinction coefficient of $8270 \text{ cm}^{-1} \text{ M}^{-1}$.

In certain studies with potent inhibitors ($K_i < 10 \text{ nM}$) where the degree of inhibition of thrombin was high, a more sensitive activity assay was employed. In this assay the rate of thrombin catalyzed hydrolysis of the fluorogenic substrate Z-GPR-afc ($K_m=27 \mu\text{M}$) was determined from the increase in fluorescence at 500 nm (excitation at 400 nm) associated with production of 7-amino-4-trifluoromethyl coumarin. Concentrations of stock solutions of Z-GPR-afc were determined from measurements of absorbance at 380 nm of the 7-amino-4-trifluoromethyl coumarin produced upon complete hydrolysis of an aliquot of the stock solution by thrombin.

Activity assays were performed by diluting a stock solution of substrate at least tenfold to a final concentration $\leq 0.1 K_m$ into a solution containing enzyme or enzyme equilibrated with inhibitor. Times required to achieve equilibration between enzyme and inhibitor were determined in control experiments. Initial velocities of product formation in the absence (V_o) or presence of inhibitor (V_i) were measured. Assuming competitive inhibition, and that unity is negligible compared $K_m/[S]$, $[I]/e$, and $[I]/e$ (where $[S]$, $[I]$, and e respectively represent the total concentrations, of substrate, inhibitor and enzyme), the equilibrium constant (K_i) for dissociation of the inhibitor from the enzyme can be obtained from the dependence of V_o/V_i on $[I]$ shown in equation 1.

$$V_o/V_i = 1 + [I]/K_i \quad (1)$$

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The activities shown by this assay indicate that the compounds of the invention are therapeutically useful for treating various conditions in patients suffering from unstable angina, refractory angina, myocardial infarction, transient ischemic attacks, atrial fibrillation, thrombotic stroke, embolic stroke, deep vein thrombosis, disseminated intravascular coagulation, and reocclusion or restenosis of recanalized vessels.

10 *In Vivo* Studies To Measure Thrombotic Occlusions

Applicants have conducted *in vivo* studies of the compounds claimed herein using the following rat ferric chloride assay.

In the assay used to determine *in vivo* activity of the thrombin inhibitors or the invention, Male Sprague-Dawley rats (body weights 200-350 grams) were anesthetized with dial-urethane solution (0.1 ml/100 gm body weight i.p.), and a lateral tail vein was cannulated with a 23 gauge needle connected to a 12 inch length of PE50 tubing. The tubing was attached to a 3-way valve by a tubing adapter. Saline (control) or test compound, as appropriate, was administered via the tail vein catheter. A tracheostomy was performed with a 0.75 inch length of PE205 tubing. The right carotid artery was exposed and a 1.3 mm diameter Doppler flow probe was placed on the vessel. Body temperature was maintained at 37°C using a heat lamp.

Rats (8-10/group) were randomized to continuous intravenous infusions of saline or test compound administered via the tail vein at a rate of 0.028 ml/min. Treatment infusions were initiated 120 min before the placement of a 3 mm square piece of Whatman No. 1 filter paper saturated with 35% FeCl₃ onto the exposed carotid artery distal to the flow probe. Treatment infusions were continued for an additional 60 minutes after the application of FeCl₃ (total infusion duration 180 minutes) if thrombotic occlusions did not occur, or were terminated 30 minutes after thrombotic occlusion of the vessel. Time to occlusion was defined as the time from application of FeCl₃ to thrombotic occlusion of the vessel. At the termination of the study (60

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minutes after application of FeCl₃ in animals which did not occlude, or at 30 minutes after thrombotic occlusion), 3 ml blood samples were drawn by cardiac puncture into 0.3 ml of 3.8% sodium citrate.

The results show that compounds of the invention prevent thrombotic occlusions.

TABLE 3
In vivo ferric chloride study measuring incidence of occlusion

10

G	Incidence of occlusion	i.v. dose (μg/kg/min)
---	------------------------	-----------------------

0/6

10

1/6

1

15

1/6

1

0/6

10

Thrombin Inhibitors - Therapeutic Uses

Anticoagulant therapy is indicated for the treatment and prevention of a variety of thrombotic conditions, particularly coronary

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artery and cerebrovascular disease. Those experienced in this field are readily aware of the circumstances requiring anticoagulant therapy. The term "patient" used herein is taken to mean mammals such as primates, including humans, sheep, horses, cattle, pigs, dogs, cats, rats, and mice.

5 Thrombin inhibition is useful not only in the anticoagulant therapy of individuals having thrombotic conditions, but is useful whenever inhibition of blood coagulation is required such as to prevent coagulation of stored whole blood and to prevent coagulation in other biological samples for testing or storage. Thus, the thrombin inhibitors
10 can be added to or contacted with any medium containing or suspected of containing thrombin and in which it is desired that blood coagulation be inhibited, e.g. when contacting the mammal's blood with material selected from the group consisting of vascular grafts, stents, orthopedic prosthesis, cardiac prosthesis, and extracorporeal circulation systems

15 The thrombin inhibitors of the invention can be administered in such oral forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixers, tinctures, suspensions, syrups, and emulsions. Likewise, they may be administered in intravenous (bolus or infusion), intraperitoneal,
20 subcutaneous, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be employed as an anti-aggregation agent. For treating ocular build up of fibrin, the compounds may be administered intraocularly or topically as well as orally or parenterally.

25 The thrombin inhibitors can be administered in the form of a depot injection or implant preparation which may be formulated in such a manner as to permit a sustained release of the active ingredient. The active ingredient can be compressed into pellets or small cylinders and implanted subcutaneously or intramuscularly as depot injections or
30 implants. Implants may employ inert materials such as biodegradable polymers or synthetic silicones, for example, Silastic, silicone rubber or other polymers manufactured by the Dow-Corning Corporation.

 The thrombin inhibitors can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large

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unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

5 The thrombin inhibitors may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The thrombin inhibitors may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxy-propyl-methacrylamide-phenol, polyhydroxyethyl-aspartamide-phenol, or
10 polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the thrombin inhibitors may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric
15 acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross linked or amphipathic block copolymers of hydrogels.

The dosage regimen utilizing the thrombin inhibitors is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the
20 condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

25 Oral dosages of the thrombin inhibitors, when used for the indicated effects, will range between about 0.1 mg per kg of body weight per day (mg/kg/day) to about 100 mg/kg/day and preferably 1.0-100 mg/kg/day and most preferably 1-20 mg/kg/day. Intravenously, the most preferred doses will range from about 0.01 to about 10 mg/kg/minute
30 during a constant rate infusion. Advantageously, the thrombin inhibitors may be administered in divided doses of two, three, or four times daily. Furthermore, they can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in

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that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regime.

5 The thrombin inhibitors are typically administered as active ingredients in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixers, syrups and the like, and consistent with convention pharmaceutical practices.

10 For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral

15 administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include

20 starch, gelatin, natural sugars such as glucose or beta-lactose, corn-sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate,

25 sodium chloride and the like. Disintegrators include, without limitation, starch methyl cellulose, agar, bentonite, xanthan gum and the like.

The thrombin inhibitors can also be co-administered with suitable anti-coagulation agents or thrombolytic agents such as plasminogen activators or streptokinase to achieve synergistic effects in

30 the treatment of various ascular pathologies. For example, thrombin inhibitors enhance the efficiency of tissue plasminogen activator-mediated thrombolytic reperfusion. Thrombin inhibitors may be administered first following thrombus formation, and tissue plasminogen

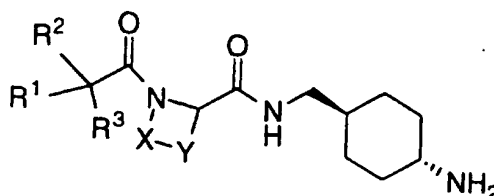
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activator or other plasminogen activator is administered thereafter. They may also be combined with heparin, aspirin, or warfarin.

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WHAT IS CLAIMED IS:

1. A compound having the formula



5 wherein

R¹ and R² are independently

hydrogen,

phenyl,

10 mono- or di-halogenated phenyl,

naphthyl,

biphenyl,

a 5- to 7- membered mono- or bicyclic heterocyclic ring or
bicyclic heterocyclic ring system any ring of which may be
15 saturated or unsaturated, and which consists of carbon atoms and
from one to three heteroatoms selected from the group consisting
of N, O and S,

C₁₋₄ alkyl,

branched C₁₋₄ alkyl,

20 C₃₋₇ cycloalkyl,

C₅₋₁₂ bicyclic alkyl,

C₁₁₋₁₆ tricyclic alkyl,

(CH₂)_nR⁴,

CH(R⁴)₂, wherein R⁴ is the same or different,

25 CH(R⁴)(OR⁴), provided that OR⁴ is not OCOOH or
OCONHR⁵,

(CH₂)_nOR⁴, provided that OR⁴ is not OCOOH or
OCONHR⁵ or

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R^2 may be joined with R^1 to form a four- to seven membered carbon ring in which zero to two carbon atoms may be substituted with heteroatoms independently selected from the list N, O, and S,

5 where n is 1, 2, 3 or 4;

R^3 is

10 H,
N(R^1)₂, wherein R^1 is the same or different,
 R^1 CONH, provided R^1 is not hydrogen,
 R^1 CONH,
(CH₂)_pOH, where p is 0, 1, 2, 3 or 4,
 R^1 SO₂NH, provided R^1 is not hydrogen, or
15 (R^1)_mNCONH, where m is 1 or 2, wherein R^1 is the same or different;

R^4 is

20 phenyl,
mono- or di-halogenated phenyl,
naphthyl,
biphenyl,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or
bicyclic heterocyclic ring system any ring of which may be
25 saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S,
COOR⁵,
CONHR⁵,
30 C₁₋₄ alkyl,
branched C₁₋₄ alkyl,
C₃₋₇ cycloalkyl,
C₅₋₁₂ bicyclic alkyl, or
C₁₁₋₁₆ tricyclic alkyl;

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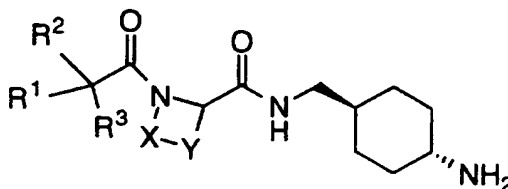
R⁵ is

hydrogen,
C₁₋₄ alkyl, or
5 branched C₁₋₄ alkyl;

X is (CH₂)_q where q is 1 or 2; or
NR¹CH₂; and

10 Y is SCH₂, or
(CH₂)_r where r is 1 or 2.

2. A compound of claim 1 having the formula:



15

wherein

R¹ and R² are independently

hydrogen,
20 phenyl,
naphthyl,
biphenyl,
a 5- to 7- membered mono- or bicyclic heterocyclic ring or
bicyclic heterocyclic ring system any ring of which may be
25 saturated or unsaturated, and which consists of carbon atoms and
from one to three heteroatoms selected from the group consisting
of N, O and S,
C₁₋₄ alkyl,
branched C₁₋₄ alkyl,
30 C₃₋₇ cycloalkyl,

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- C5-12 bicyclic alkyl,
C11-16 tricyclic alkyl,
(CH₂)_nR⁴,
CH(R⁴)₂, wherein R⁴ is the same or different,
5 CH(R⁴)(OR⁴), provided that OR⁴ is not OCOOH or
OCONHR⁵,
(CH₂)_nOR⁴, provided that OR⁴ is not OCOOH or
OCONHR⁵ or
10 R² may be joined with R¹ to form a four- to seven membered
carbon ring in which zero to two carbon atoms may be substituted
with heteroatoms independently selected from the list N, O, and S,

where n is 1, 2, 3 or 4;
R³ is
15 H,
N(R¹)₂, wherein R¹ is the same or different,
R¹OCONH,
R¹CONH,
20 (CH₂)_pOH, where p is 0, 1, 2, 3 or 4,
R¹SO₂NH, or
(R¹)_mNCONH, where m is 1 or 2, wherein R¹ is the same or
different;

25 R⁴ is

phenyl,
naphthyl,
biphenyl,
30 a 5- to 7- membered mono- or bicyclic heterocyclic ring or
bicyclic heterocyclic ring system any ring of which may be
saturated or unsaturated, and which consists of carbon atoms and
from one to three heteroatoms selected from the group consisting
of N, O and S,

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5 C₁₋₄ alkyl,
branched C₁₋₄ alkyl,
C₃₋₇ cycloalkyl,
C₅₋₁₂ bicyclic alkyl, or
C₁₁₋₁₆ tricyclic alkyl;

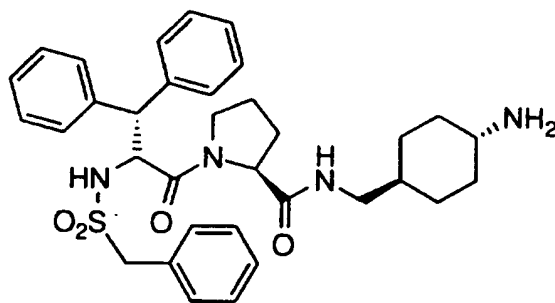
X is (CH₂)_q where q is 1 or 2; or
NR¹CH₂; and

10 Y is SCH₂, or
(CH₂)_r where r is 1 or 2.

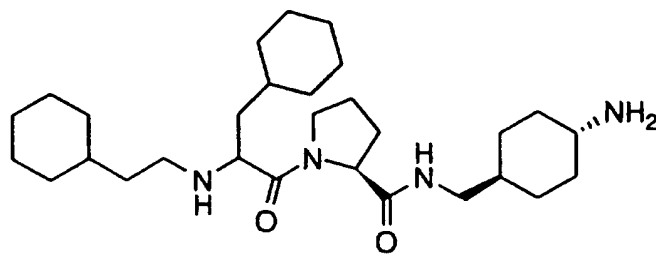
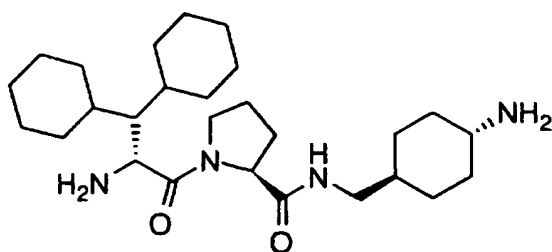
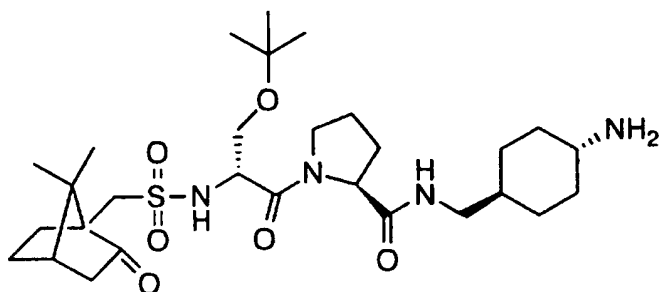
15 3. A compound of Claim 2 wherein X is (CH₂)_q; q is 1;
Y is (CH₂)_r; and r is 1 or 2.

4. A compound of Claim 2 wherein X is NR¹CH₂; R¹ is
hydrogen or C₁₋₄ alkyl; Y is (CH₂)_r; and r is 2.

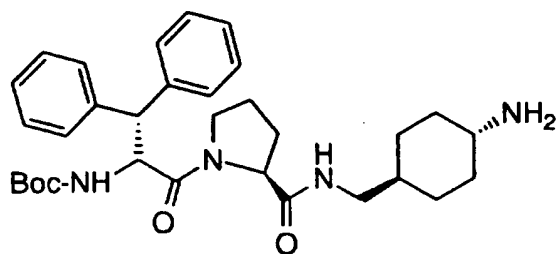
20 5. A compound of claim 3 selected from the group
consisting of:



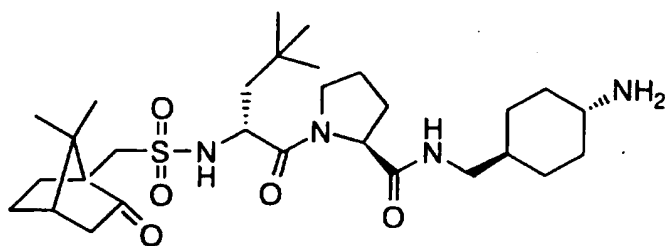
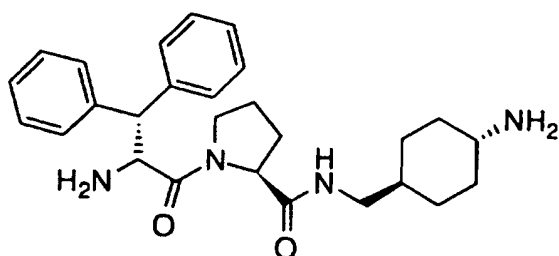
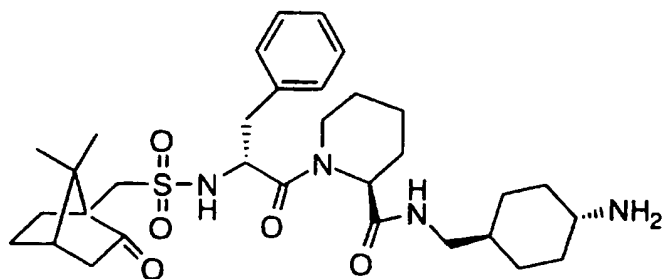
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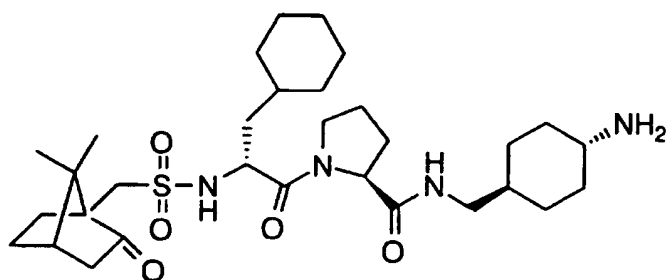
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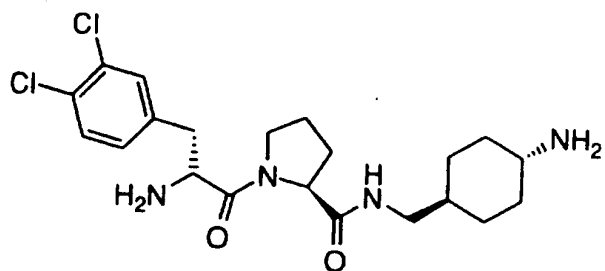
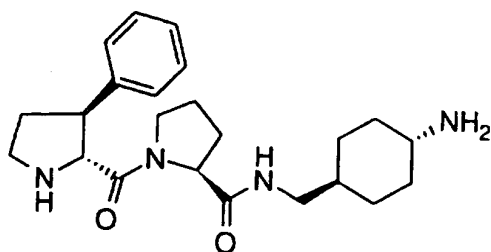
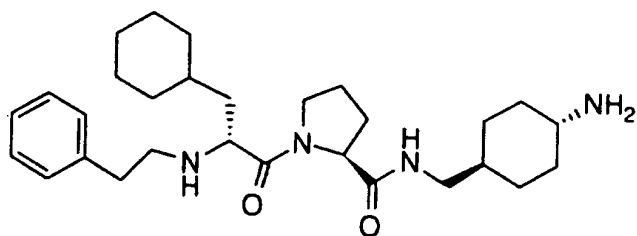
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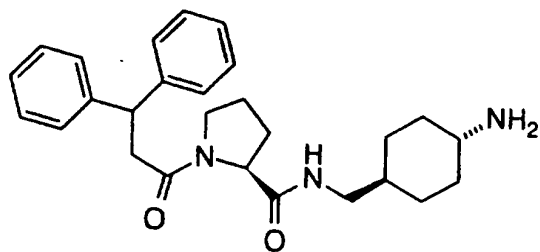
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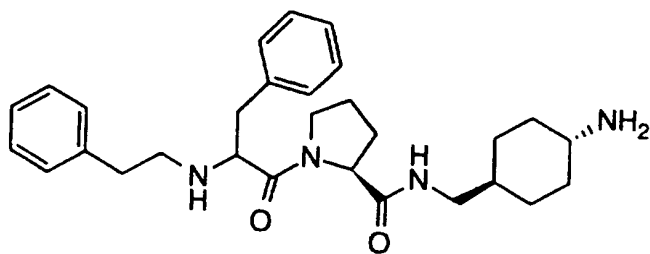
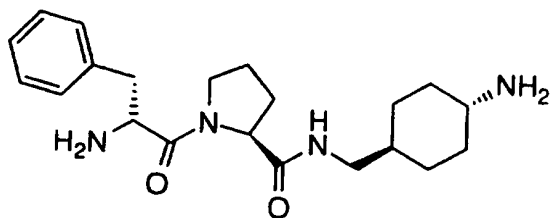
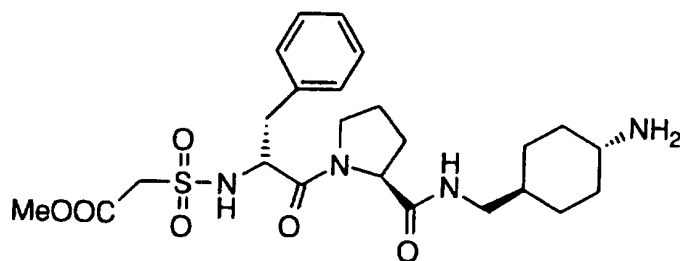
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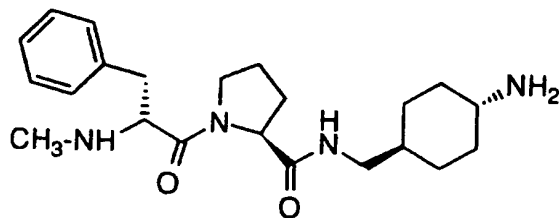
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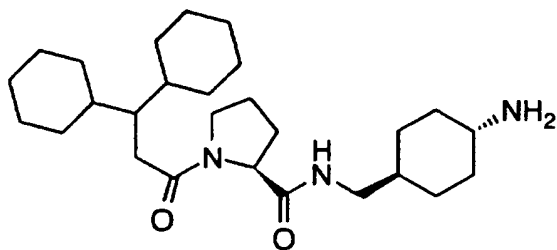
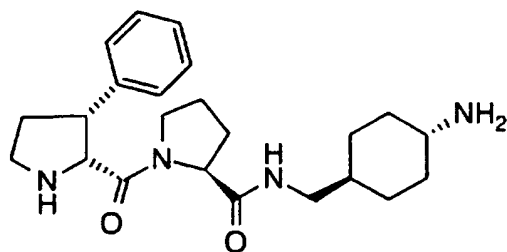
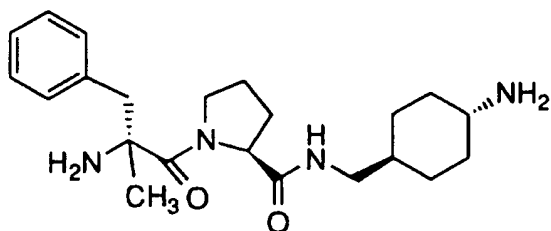
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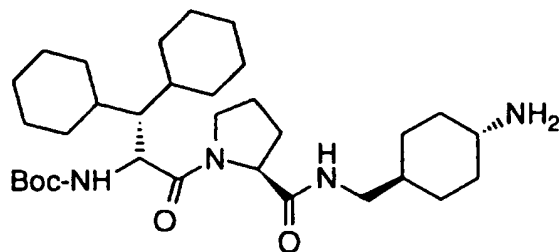
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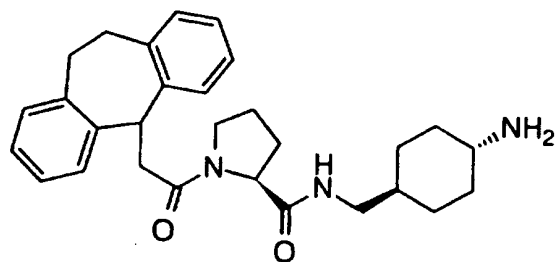
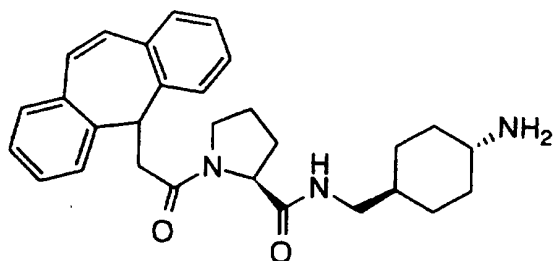
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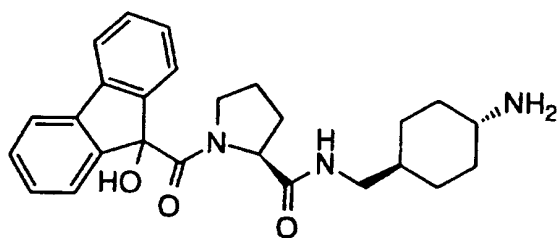
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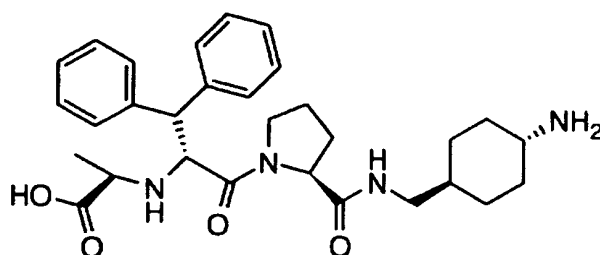
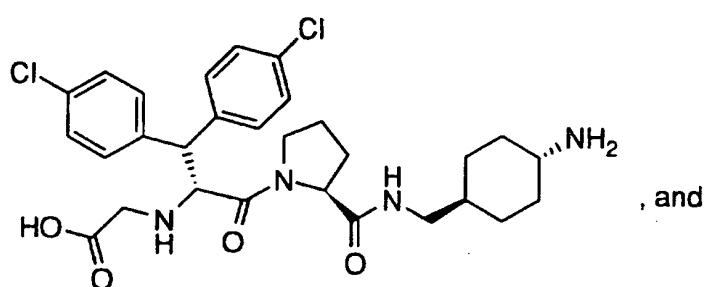
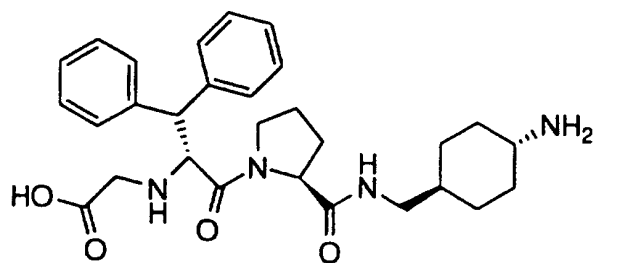
, and



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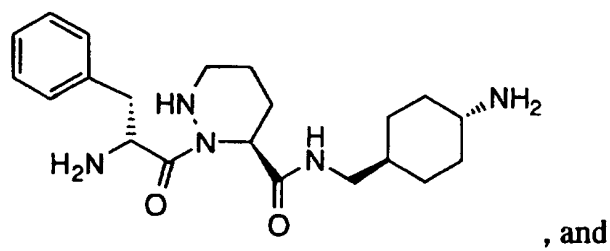
6. A compound of claim 1 selected from the group consisting of:

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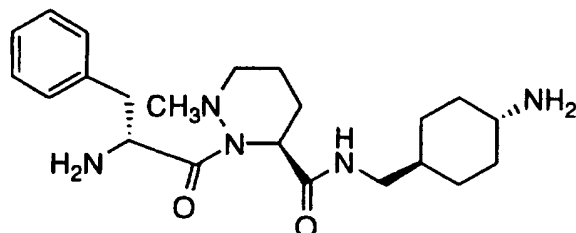


7. A compound of claim 4 selected from the group consisting of:

5



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8. A composition for inhibiting thrombin in blood comprising a compound of Claim 1 and a pharmaceutically acceptable carrier.

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9. A composition for inhibiting formation of blood platelet aggregates in blood comprising a compound of Claim 1 and a pharmaceutically acceptable carrier.

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10. A composition for inhibiting thrombus formation in blood comprising a compound of Claim 1 and a pharmaceutically acceptable carrier.

11. A method for inhibiting thrombin in blood comprising adding to the blood a composition of Claim 8.

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12. A method for inhibiting formation of blood platelet aggregates in blood comprising adding to the blood a composition of Claim 9.

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13. A method for inhibiting thrombus formation in blood comprising adding to the blood a composition of Claim 10.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09007

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07D 207/06, 211/08, 237/04; A61K 31/40, 31/50, 31/445

US CL : 544/224; 546/226; 548/518, 537; 514/247, 354, 422, 423

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/224; 546/226; 548/518, 537; 514/247, 354, 422, 423

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN CAS ONLINE, APS (THROMBIN AND THE DIFFERENT CLASSES)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	EP, A, 0,648,780 (BRISTOL-MYERS SQUIBB COMPANY) 19 April 1995, see pages 3 and 4.	1-13
A	US, A, 2,277,409 (MURRAY) 24 March 1942, see column 1, lines 1-36.	1-7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Special categories of cited documents:	
A document defining the general state of the art which is not considered to be of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O document referring to an oral disclosure, use, exhibition or other means	*Z* document member of the same patent family
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 SEPTEMBER 1995

Date of mailing of the international search report

29 SEP 1995

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